SOLUTE-SOLVENT INTERACTION FREE ENERGIES AND RETENTIVITY OF REVERSED PHASE LIQUID CHROMATOGRAPHY COLUMNS

BY

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Recently, a description of the molecular mechanism of retention in reversed phase liquid chromatography (RPLC) was derived by using a partition model and statistical mechanical theory. This theory relates the capacity factor, k', of a solute in RPLC to the binary interaction constant between the solute molecules and the mobile phase molecules. The binary interaction constant is related to the interaction free energy through the Boltzmann's constant and the absolute temperature. Solute-solvent interaction free energies can be obtained from chromatographic data if the partition theory of solute retention is correct.

We have tested the validity of the predictions from this theory by using more than 300 sets of retention data from the literature and our laboratory. With only a few exceptions, the data confirm all the predictions, and the solute-solvent interaction free energies are found to be solute size dependent, which was a case not considered when the

theory was first developed.

Although RPLC is one of the most popular separation techniques, the actual effect that the stationary phase has on solute retention is still not completely known. Much research has been done on the role and effects of the mobile phase on solute retention in RPLC, but there has been a lack of systematic approaches in characterizing RPLC columns.

We have developed a simple method to classify RPLC columns in accordance with their retentivity by using more than 20 solutes of very different chemical structures. The solvent strength of the mobile phase has to be accounted for before the retentivity of these columns can be investigated. This is accomplished by plotting ln k' of the solute against the $E_T(30)$ polarity values of the mobile phase. A good parameter that sums all the retention due to the stationary phase is the capacity factor of the solute at 100% water, ln k_w. By utilizing ln k_w and the slope from the $E_T(30)$ plot, a relative retentivity of RPLC columns is obtained.

CHAPTER I
INTRODUCTION

Reversed phase liquid chromatography (RPLC) is the most common mode of high-performance liquid chromatography used today. RPLC is estimated to account for more than 80% of chromatographic systems currently employed (Melander and Horvath, 1980). The name "reversed phase" was introduced by Howard and Martin (1950) when they used non-polar liquid paraffin and n-octane as the stationary phase to separate fatty acids. They used the term to distinguish it from "normal phase" chromatography, which consists of a polar stationary phase and a non-polar mobile phase. The use of RPLC has shown an overwhelming increase when columns packed with chemically stable microparticulate-bonded silica became available. The practical use of commercial bonded phase packings in RPLC can be dated back to Kirkland and DeStefano (1970).

These bonded phases add great advantages to RPLC, including the fact that aqueous mobile phases having low toxicity and high optical transparency can be adopted to achieve most separations. Also, the stability of these bonded phases are superior to other stationary phases. Aqueous mobile phases of pH between 7.5 and 2.5 are compatible with these bonded phases. Moreover, because of the wide range of polarity of the mobile phases that can be used with these bonded phases,

an enormous amount of chemical compounds can be separated by using bonded phases in RPLC.

Despite the prevalence of RPLC in analytical separations, there is a lack of full understanding of solute retention and selectivity. This lack of understanding hinders the development of a retention index in RPLC similar to the Kovats index (Kovats 1965) or Rohrschneider and McReynolds (Rohrschneider 1965; McReynolds 1970) constants in gas chromatography (GC). It also creates problems for practical chromatographers in developing separation schemes and for comparison of retention results obtained from different laboratories. Horvath and coworkers (Horvath et al., 1976; Melander and Horvath 1980) explained their solvophobic retention mechanism for RPLC using a solute association model. This is an important step toward the deconvolution of solute retention on the molecular basis in RPLC, although there are many shortcomings in this theory such as the ignorance of the retention effects due to the stationary phase. The deficiencies of this theory have been demonstrated in the literature by many researchers (Lochmüller and Wilder, 1979; Lochmüller et al., 1981; Sadek and Carr, 1984; Berendsen et al., 1980; Sentell and Dorsey, 1989a). Dill and coworkers (Dill 1987; Marqusee and Dill, 1986a; Dorsey and Dill, 1989) have put forth a molecular basis retention mechanism for small solutes in RPLC using a partition model. In the following sections, these two significant retention mechanisms in RPLC will be discussed briefly.

Solvophobic Retention Mechanism

The solvophobic theory was promoted by Horvath and coworkers (Horvath et al., 1976; Melander and Horvath, 1980) utilizing the

solvophobic interaction of solute association from Sinanoglu (1967). In this theory, solute retention is viewed as a reversible association of the solute with the hydrocarbonaceous ligands of the stationary phase. The hydrophobic interactions between the solute and the stationary phase are thought to come from the fact that the mobile phase in RPLC is relative polar, and the nonpolar moiety of the solute is repelled and forced to associate with the nonpolar stationary phase. As Melander and Horvath (1980) stated, the solute retention in solvophobic theory actually involves two steps. The first step is the opening of a cavity in the solvent that has the same size and shape as the solute molecule. The second step is the placement of the solute in the solvent cavity and all the interactions between the encircled solute and the solvent molecules are followed. These interactions are originated from the van der Waals forces and the electrostatic interactions amid the solute and the solvent molecules.

The free energy associated with the first step of the solvophobic theory can be expressed as

$$\Delta G_e = \kappa^e(r)\ A\ \gamma\ N \qquad (1\text{-}1)$$

where N is Avogadro's number, γ is the surface tension of the solvent, A is the molecular surface area of the solute and $\kappa^e(r)$ is a proportional factor for the cavity size. From equation 1-1, the free energy of the solvent cavity is proportional to the surface tension of the solvent. The free energy related to solute-solvent interactions is comprised of two chemical effects and an entropic term

$$\Delta G_{int} = \Delta G_{vdw} + \Delta G_{es} + RT \ln(RT/PV) \qquad (1\text{-}2)$$

The change in free energy from the first chemical effect, ΔG_{vdw}, can be calculated by using van der Waals potential in condensed media for the solute and solvent. The change in free energy from the second chemical effect, ΔG_{es}, can be further divided into dipole interactions and ionic interactions. The dipole interactions can be treated according to the Onsager reaction field approach (1936) while the ionic interactions can be estimated using the Debye-Huckel theory. The entropic term in equation 1-2 is related to the "free volume" of the solute and this free volume is the measure of the volume of a molecule before it collides with another molecule. The solute's molar volume can be used to compute the free volume (Melander and Horvath 1980).

As far as the stationary phase effects on the retention of solutes, Melander and Horvath (1980) considered them negligible because the stationary phase is nonpolar and the only attraction between the solute and the stationary phase is van der Waals forces. This van der Waals force is insignificant compared to the van der Waals interactions between the solute and solvent molecules. Although Melander and Horvath (1980) acknowledged that an entropic term can be used to sum up the restricted translational freedom of the bonded hydrocarbonaceous ligands at the silica surface, they elected to pay no attention to this term in their theory. In summary, the solvophobic retention mechanism takes the approach that solute retention in RPLC is largely due to the hydrophobic interactions of the solute and solvent molecules. The stationary phase

is treated as a passive entity that is forced to receive the solute from the solvent, and its contribution to solute retention is negligible.

Partition Retention Mechanism

One of the catastrophic downfalls of the solvophobic theory is that it disregarded the effect of the stationary phase on solute retention. Dill and coworkers (Dill 1987; Marqusee and Dill, 1986a, Dorsey and Dill, 1989) applied the mean-field statistical mechanical theory, lattice theories (Hill 1986) and random-mixing approximation to explore the retention mechanism of RPLC on the molecular level. Dill and Dorsey (1989) used a simple partition model and took into account the stationary phase effect on solute retention. They assumed the stationary phase in RPLC as an "interphase" and solute retention is due to partition between the the bulk mobile phase and the interphase. In their model, the dominating driving force of solute transfer is from the differences amid the chemical affinity of the mobile and stationary phase. The solute capacity factor measured in RPLC can be expressed as

$$k' = K \Phi \qquad (1\text{-}3)$$

where k' is the capacity factor of the solute, K is the equilibrium constant of the partition process and Φ is the phase ratio of the chromatographic system and in RPLC, this is defined as the ratio of the volumes of stationary and mobile phases. The equilibrium constant, K, can be related to the free energy G(S) for the solute S

$$\ln K = -(G^{\circ}_{sta}(S) - G^{\circ}_{mobile}(S))/kT \qquad (1\text{-}4)$$

where $G^{\circ}_{sta}(S)$ and $G^{\circ}_{mobile}(S)$ are the standard-state free energies of solute S in the stationary phase and the mobile phase respectively, and kT is the Boltzmann's constant multiplied by absolute temperature.

The actual solute transfer of this partition process is comprised of three steps. First, a cavity having the same size as the solute molecule is opened in the stationary phase. Second, the solute molecule from the mobile phase is transferred into the stationary phase cavity. Third, the cavity left behind by the solute molecule in the mobile phase is filled by other mobile phase molecules. These three steps are shown in Figure 1-1, and for simplification, the mobile phase is considered to be a single component. This will be used throughout the following discussion of this retention mechanism. For a detailed derivation of this retention mechanism using a binary mobile phase, the readers should refer to Dill (1987).

The free energies involved in these three steps can be calculated using pair interactions of molecules. The pair potential, $u(r^*)$, of bringing a spherical molecule X from an infinite separation to within an average equilibrium separation of spherical molecule Y can be defined as

$$u(r^*) - u(\infty) = u(r^*) = w_{XY} \qquad (1\text{-}5)$$

where w_{XY} is the reversible work. Figure 1-2 shows a pair potential, with short-ranged repulsion and longer-ranged attraction. The partition process in RPLC is the result of this pair potential. The exact nature of the pair potential is difficult to identify since the partition coefficient of the process alone does not give enough information, but

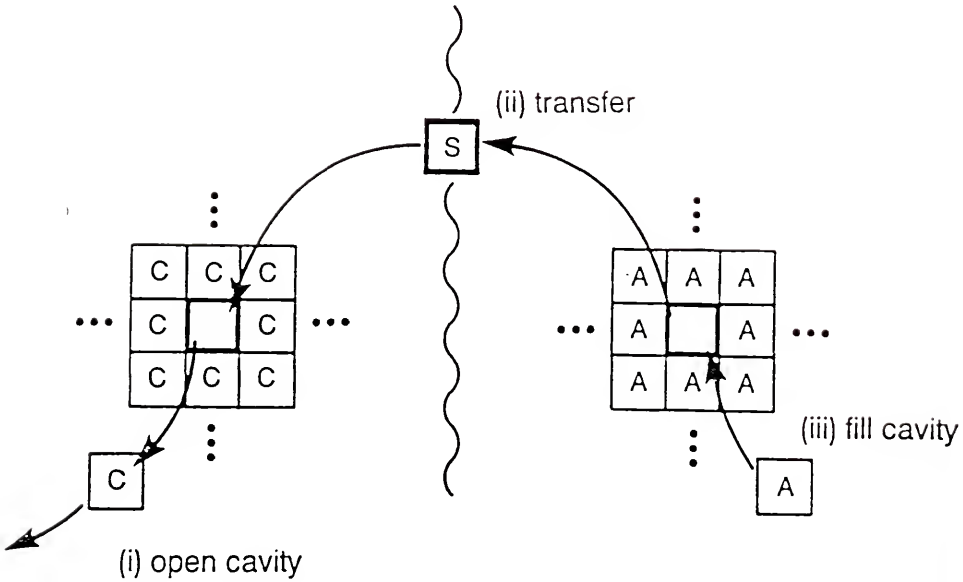


Figure 1-1. The three steps involved in the partition process where the transfer of solute molecule S requires the opening of a cavity in the stationary phase C and the closing of a cavity in mobile phase A (Dorsey and Dill, 1989).

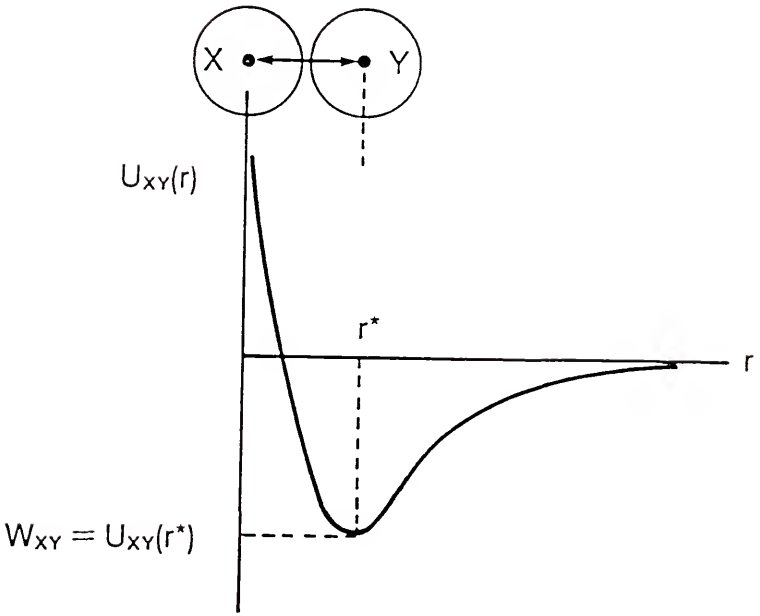


Figure 1-2. Pair interaction potential, $u_{XY}(r)$, for two simple molecules. Reversible work for bringing molecules X and Y together to their equilibrium separation r^* is w_{XY} (Dorsey and Dill, 1989).

it is generally believed that it is related to hydrogen bonding, dipole, ionic and van der Waals interactions.

Dill and coworkers (Dill 1987; Marqusee and Dill, 1986a; Dorsey and Dill, 1989) stated that from the lattice theory of liquids, the opening of the cavity in the stationary phase leaves z sides, or z/2 contact (pair of sides), without contact. The coordination number, z, is the number of neighbors of each molecules, or of each lattice site. This cavity opening process in the stationary phase contributes $(-z/2)w_{CC}$ to the free energy of the overall process. The same argument can be applied to the closing of the cavity in the mobile phase, and therefore the free energy contributed is $(-z/2)w_{AA}$. The solute molecule, S, in the mobile phase is surrounded by z nearest-neighbors of the mobile phase molecules(solute self-association is ignored here). The transfer process of the solute from the mobile phase to the stationary phase involves the formation of z number of contacts in the stationary phase while breaking z number of contacts in the mobile phase. The free energies attributed from these two processes are zw_{SC} and zw_{SA} respectively. The overall free energy of transfer is shown below.

$$\Delta G_{transfer} = z(w_{SC} - w_{SA} + w_{AA}/2 - w_{CC}/2) \qquad (1\text{-}6)$$

It is more convenient to express this free energy of transfer in terms of the binary interaction constant, χ_{SC} and χ_{SA},

$$(\Delta G_{transfer})/kT = \chi_{SC} - \chi_{SA} \qquad (1\text{-}7)$$

where kT is Boltzmann's constant multiplied by the absolute temperature. The binary interaction constant is defined as

$$\chi_{XY} = z/kT \left(w_{XY} - (w_{XX} + w_{YY})/2 \right) \qquad (1\text{-}8)$$

Equation 1-6 can be further simplified by using the Hilderbrand solubility parameter concept (Hilderbrand and Scott, 1950) for small molecules. The major interactions for small molecules are the induced dipoles and the binary interaction constant can be approximated as a product of factors involving unitary interaction constants, δ_X and δ_Y, the solubility parameters

$$\chi_{XY} = \text{constant} \left(\delta_X - \delta_Y \right)^2 \qquad (1\text{-}9)$$

The solubility parameter of a compound can be measured from the enthalpy of vaporization of the pure compound. Although solubility parameters have been used to model chromatographic retention (Schoenmakers et al., 1978; 1982; 1983; Tijssen et al,. 1976; Karger et al., 1978) and the factorization can reduce the size of the data base of constants, this approximation is generally poor compared to the binary interaction constant except in the case when dispersion forces are the principle interactions (Schoenmakers et al., 1978).

The underlying molecular interactions that drive this partition process in RPLC are very complicated. Since the capacity factor of a

solute in RPLC only gives information on the equilibrium constant of the partition process, other experimental data are needed to fully understand these interactions (Dorsey and Dill, 1989). For example, the entropic dependence of these interactions can be determined by measuring the relationship between temperature and the capacity factor of the solute. The variation of the capacity factor with pH and salt concentration in the mobile phase can be used to uncover the effects of the electrostatic interactions on the partition process.

The partition model is supported by many experimental findings. First, the capacity factors of solutes in RPLC are found to be directly proportional to the water/octanol partition coefficients (Schantz and Martire, 1987; Opperhuizen et al., 1987; Braumann 1986; Minick et al., 1987; Kaliszan 1986). Second, inasmuch as the cavity formation and closing in the stationary and mobile phases are important, the capacity factors of solutes in RPLC are linearly dependent on the size of the solute (Colin et al., 1983; Mockel et al., 1987; Horvath et al., 1976). Third, the partition of solutes into the stationary phase increases when the solubility of the solutes decreases in the mobile phase and therefore the retention of the solutes increases. This is evidenced when salt content in the mobile phase is found to be directly proportional to the retention of hydrocarbons in RPLC (Tanford 1980; Melander and Horvath 1980). Fourth, the surface tension, γ_A, of a pure mobile phase can be expressed as

$$\gamma_A \approx w_{AA}/2a \qquad (1\text{-}10)$$

where a is the area per AA contact. Although it only describes the cavity in the mobile phase, at situations when the stationary phase effects on the solute retention are small (i.e., the other terms in equation 1-6 are small) the surface tension of the mobile phase should be proportional to the retention (Melander and Horvath, 1980; Hammer et al., 1982; Horvath et al., 1976).

Perhaps another possibility of solute retention in RPLC is by adsorption. An adsorption mechanism related to other types of chromatography has been developed by Martire and Boehm (1983) and Jaroniec (1984). Dill (1987) has examined the possibility of adsorption mechanism and compared it with the partition theory that he proposed. He concluded that the adsorption model is inferior to the partition model for two reasons. First, in the adsorption model, the density of the grafted alkyl chains on the stationary phase should have no effect on the retention of solutes. On the other hand, in the partition model, the partitioning of solutes should decrease if the density of the grafted alkyl chains increases because it would be entropically expensive for the grafted chain to uptake the solutes after a critical bonding density (Dill 1987; Dorsey and Dill, 1989). This decrease in solute partitioning with increase in alkyl chain density has been reported (Claudy et al., 1985; Sentell and Dorsey, 1989a). Second, if partition is the predominant mechanism of solute retention, the ln k's of a homologous series should be a linear function of the logarithm of the appropriate water/octanol partition coefficient, with a slope of 1. For the adsorption mechanism, such a plot should have a slope of 1/z. This is because in adsorption, only one side of the solute is in contact with the alkyl chains, and therefore the transfer free energy of this

process is only 1/z amount of the partition process measured by the water/octanol partition coefficient. In the partition model, the solute is embedded by the alkyl chains, and the whole solute is in contact with the alkyl chains. This simulates the partition process between two bulk liquid phases, and hence the ln k' of a solute in RPLC has a one-to-one relationship with the corresponding logarithm of the water/octanol partition coefficient, and this has been found in many systems (Butte et al., 1981; Braumann 1986; Kaliszan 1986).

Although Dill and coworkers (Dill 1987; Marqusee and Dill, 1986a; Dorsey and Dill, 1989) used a few assumptions and some simple approximations on the partition mechanism, their model correctly accounted for most of the experimental observations that cannot be explained sufficiently by other retention mechanisms. This model does not treat the stationary phase as an inert part of the chromatographic separation. The stationary phase is considered to play an active role in the solute partition process since the free energy involved in the cavity opening procedure in the stationary phase is of significant influence on the total energetic of the chromatographic separation in RPLC.

Solute-Solvent Interaction Constant

The binary interaction constant χ is an important parameter for the partition mechanism and can be used to calculate the solute-solvent free energy as seen in equation 1-7. The binary interaction constant χ is usually obtained by measuring the free energy of transfer or vapor pressure in the Henry's law region (Hill 1986). Dill (1987) used the partition model and predicted that in an RPLC system having a binary

mobile phase, the capacity factor of a solute can be estimated by using the binary interaction constants among the solutes and solvents. He calculated the capacity factors of some solutes by using the χ obtained in vapor pressure measurement for the binary solvents and χ obtained from free energy of transfer of the solutes and found good agreement with experimental data. The scenario can be reversed by using chromatographic capacity factors of solutes to evaluate the solute-solvent interaction constant. This should be very beneficial because chromatographic data are more convenient to collect and reliable than vapor pressure measurement data.

Stationary Phase in RPLC

The retention mechanism in RPLC has been thoroughly reviewed and it has been pointed out by the partition mechanism that the stationary phase in RPLC contributes a great deal to solute retention and cannot be ignored. In order to fully understand the chromatographic process and to forge ahead the applicability of RPLC, the structure and properties of stationary phases used in RPLC deserve an in-depth investigation. The stationary phase in RPLC has not been under the intense study as the mobile phase has, but recently a few articles have been published on the structure and properties of them (Sander et al., 1983; Gilpin 1984; Carr and Harris, 1986; Buszewski and Suprynowicz, 1987; Nawrocki and Buszewski, 1988).

Silica is by far the most common base material used in RPLC stationary phases because silica is the best-known inorganic, polymeric material (Unger 1979). Many reliable methods are available to control the size and pore size of silica, and silica of the required size and

shape can be produce in large quantity. Before the introduction of chemically bonded phases, silica was used as the support for liquid-liquid chromatography where a thin film of liquid is coated onto the surface of the silica and then is used as the stationary phase (Unger 1979). These liquid stationary phases suffer from instability and irreproducibility. The evolvement of chemically bonded phases by chemically treating the silica surface with silanes began in the middle of 1960s. They were debuted in gas chromatography as nonpolar stationary phase obtained by reacting silica gel with hexadecyltrichlorosilane (Abel et al., 1966). The employment of chemically bonded phases in liquid chromatography was first suggested by Stewart and Perry (1968). Chemically bonded phases have excelled to become the most frequently used stationary phases in RPLC today due to the advancement of synthetic technology for preparing these phases.

The underlying properties of these chemically bonded phases depend on the physical and chemical properties of the base silica, the bonding reactions and reagents used. The base silica constitutes the major portion of the stationary phase, and the first property of the base silica that influences the chromatographic process is the particle size of the silica. The size of most silica particles employed in RPLC stationary phases are in the range of 3-10 μm. The actual effect of the silica particle size on RPLC is inconclusive since inconsistent results have been reported in the literature. Dewaele and Verzele (1983) have done a study on the influence of silica particle size distribution on the reversed phase packings, and they found that the particle size affects chromatographic performance of the packings minimally. Gazda et al. (1980) also had the same conclusions, but Bristow (1978) has shown

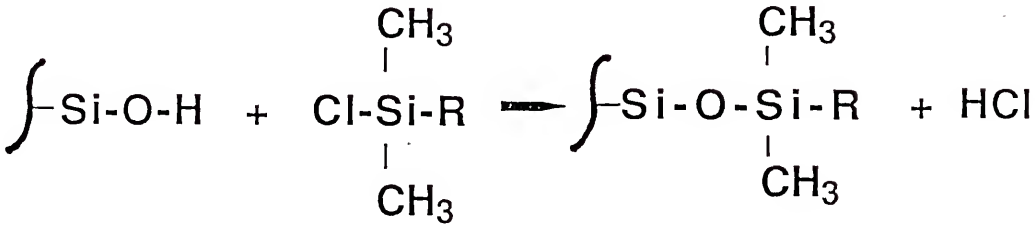
otherwise. Pore size distribution is another important physical property of silica particles. Verzele et al. (1985) have noticed that the mean pore diameter of the silica used as chromatographic packings should be in the range of 6-10 nm, and the narrower the distribution curve the better it is. Micropores of diameter 2 nm or smaller are undesirable because they can alter the final coverage of the bonded phase (Berendsen et al., 1980; Engelhart et al., 1982).

Apart from the physical properties, the surface chemistry of the silica particles endure the final characteristic of the bonded phase. Silica surface is very complex, and it has been found to consist of various kinds of silanols and siloxane bonds. The siloxane bonds are usually considered as rather chemically inert and therefore do not take part in the formation of the bonded phase. Single, geminal and vicinal forms of silanols are found on the surface of silica (Unger 1979; Majors 1980), and chromatographers have mixed feelings toward these surface silanols. First of all, these silanols react with silanes to form the bonded phase, but on the other hand, these silanols are one of the major contributions to the variability of the bonded phases. Moreover, these silanols account for the pH of the silica. Different values of pK_a of silica have been reported in the literature (Walling 1950; Karger et al., 1980; Majors 1980). Engelhardt and Müller (1981) first observed the divergence of surface pH of silica packings for chromatographic purpose. This reflects the different methods of manufacturing silicas and connects to the different chromatographic properties of silicas (Hansen et al., 1986).

Trace metal impurities found in silica is another factor which influences the property of the bonded phase. The effect of trace metal

impurities have been ignored by many researchers, but Marshall et al. (1986; 1987), Nawrocki (1986; 1987) and Sadek et al. (1987) have shown that these impurities can indeed affect the chromatographic process. Verzele et al. (1979) have pointed out that numerous transition metals can be found in the silicas that are used for chromatographic packings. Sadek et al. (1987) described that silica can contain metals in three forms: surface species, internal and secluded. The surface metals can form surface metal hydroxides which can alter the surface pH of the silicas, while both the surface and the internal metals can participate in coordination with the solute or solvents using their orbitals. The secluded metals are too far away from the surface silanols and therefore do not exert any effect on the chromatographic process. According to Verzele and coworkers (Verzele et al., 1979; Verzele and Dewaele, 1981; Verzele 1983) and Sadek et al. (1987), by acid treatment of the silica, most of the metal impurities can be leached.

Chemically bonded phases are silica particles having their surface derivatized with alkyl chains or other functionalities such as cyano or amino groups (Dorsey and Dill, 1989). The surface of the silica particles can be derivatized by reacting with organochlorosilanes or organoalkyoxysilanes, and the most common synthetic scheme is called "monomeric reaction". In this type of reaction, a single siloxane linkage is formed between one silanol on the surface of the silica and a monochlorosilane or monoalkoxysilane. A simplified diagram showing this type of monomeric reaction is depicted in Figure 1-3. The popularity of monomeric reaction is due to the fact that it gives a uniform and well-defined single layer coverage of the surface of the silica, and also the reproducibility is above other types of reactions. The maximum



R = Ligands of different functionality

Figure 1-3. Generalized bonding scheme for the synthesis of monomeric-bonded phase using a monochlorosilane.

number of silanols on the surface of silica are predicted to be about 8 $\mu mol/m^2$ and only these silanols can react with the silanes (Unger 1979). The most common C_8 and C_{18} containing silanes usually produce a surface coverage of approximately 2.5-3.0 $\mu mol/m^2$. This means that there is an immense amount of residual silanols which does not react with the silanes. These residual silanols are accessible to solutes during the chromatographic process and can cause adsorption and mixed retention mechanism. A second bonding procedure is usually employed by most manufacturers to try to minimize the residual silanols. The second bonding procedure often makes use of trimethylsilanes to react with the remaining silanols and is termed "end-capping" (Lochmüller and Marshall, 1982; Sadek and Carr, 1983; Evans et al., 1980; Dewaele et al., 1982; Marshall et al., 1987). Other methods include using silica pretreatment (Gobet and Kovats, 1984; Marshall et al., 1984), and the use of more reactive silanes have been reported (Buszewski et al., 1987; Lork et al., 1986).

Much research has been done on improving the synthetic means to increase the surface coverage or bonding density of the monomeric bonded phase (Buszewski et al., 1987; Golding et al., 1987; Khong and Simpson, 1987; Sentell et al., 1988). This striving for higher bonding density originated as a way to suppress the residual silanols, but it was soon noticed that there are other advantages to high bonding density. First, by increasing the bonding density, less silanols are exposed to the solutes and thus eliminates most of the adsorption mechanism due to the silanols. Second, the stability of the stationary phase can be raised by higher bonding density because the dense alkyl chains protect the silica from being hydrolyzed.

When a di- or trifunctional silane is used instead of a monofunctional silane in the bonding process, a polymeric coverage frequently results. The polymeric network is a consequence of the reaction among one or more of the leaving groups of the silane and the silanols on the silica surface. Sander and Wise (1984) have noted that under a controlled manner, polymeric phases can be very reproducible and have some chromatographic advantages. Wise and Sander (1985) showed that these polymeric phases have shape selectivity on polycyclic aromatic hydrocarbons and are similar to liquid crystalline phases used in GC. They proposed that polymeric phases are more ordered than monomeric phases.

The microscopic structure of the bonded phase is very important to the retention and selectivity behavior of the bonded phase. Many research groups have tried many different experimental approaches to gain better understanding of the nature and molecular organization of the alkyl chains that have been grafted onto the silica. In the early stage of the bonded phase, a simplified model was suggested and is shown in Figure 1-4 (Melander and Horvath, 1980). This model is not very realistic because it assumes that the alkyl chains are stiff rods without any internal degrees of freedom. This is contradictory to the corresponding disorder structure of alkane at the temperature of interest for chromatography. Also, the "fur" and "stack" models in Figures 1-4b and 1-4c imply that the alkyl chains are exposed to the mobile phase, but the mobile phase used in RPLC is of very high aqueous content and the hydrophobic effect should prohibit this total exposition of the nonpolar alkyl chains to the polar mobile phase (Dill 1987).

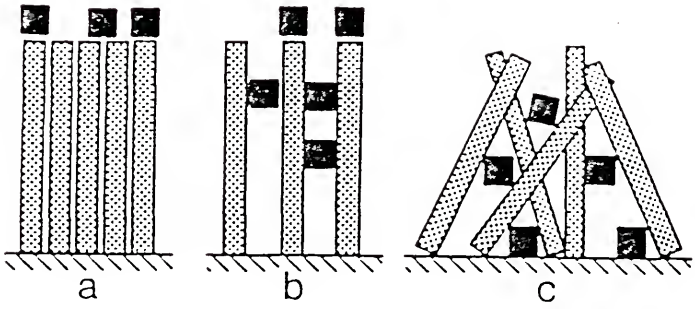


Figure 1-4. Early models of molecular structure and organization of the bonded phase in RPLC: a) "picket fence"; b) "fur"; c) "stacks" (Dill 1987).

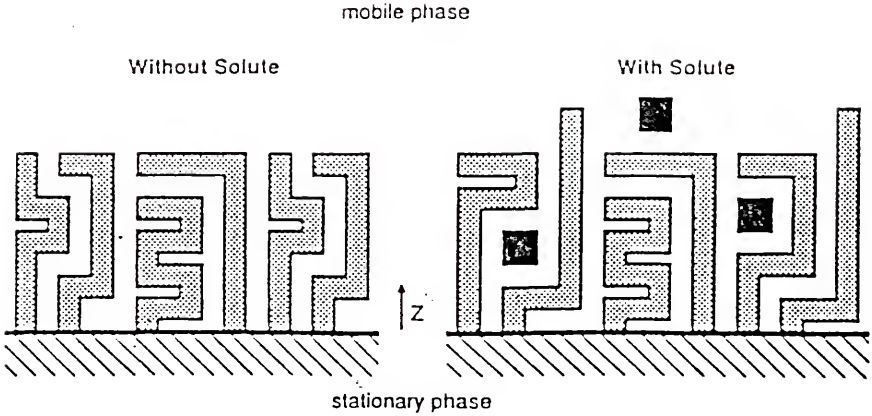


Figure 1-5. Interphase model of the bonded phase in RPLC proposed by Dill (1987).

Dill and coworkers (Dill 1987; Marqusee and Dill, 1986a; Dorsey and Dill, 1989) have proposed an interphase model for the structure of the bonded phase. Figure 1-5 is a simple representative of this model. The alkyl chains that are bonded onto the surface should have the same molecular structure and organization of other similar interfacial phases involving chain molecules such as micelles, surfactant monolayers, bilayers and microemulsions (Dill and Flory, 1980; 1981; Dill et al., 1984). A typical interphase has one side of the chain molecules anchored to an interface, and the disorder of the chains increases with the distance from the interface (Dill et al., 1988; Brown 1984; Cabane 1977). The bonded phase in RPLC has these characteristics because one side of it is the siloxane bond between the alkyl chains and the surface silanol. Gilpin and coworkers (Gilpin and Gangoda, 1983; 1984; Gilpin 1984) have shown by using NMR measurements of the T_1 of ^{13}C labelled alkyl chains that segmental motion of the alkyl chains is a function of distance from point of surface attachment. Sander et al. (1983) used fourier transform infrared spectroscopy to study a series of dimethyl-n-alkyl bonded phases ranging from C_1 to C_{22}. Their results showed that these systems have disordered chains with kinks and bends.

The molecular organization of the interphase is dominated by three factors (Dill and Flory, 1980; 1981; Dill et al., 1984; Marqusee and Dill, 1986b). First, the molecular organization of the interphase is restricted by the geometry of the interface and the density and length of the chain molecules. Second, under a poor wetting agent, the interphase will try to reject most of the hostile solvent molecules. An example is when alkyl stationary phase of RPLC is under aqueous mobile

phase. Third, in accordance with the attempt to gain maximum entropy, the interphase always adopts as much disorder as possible within all the constraints.

The interphase model of the stationary phase is very appropriate with the partition mechanism of solute retention. In theory, the partition of a solute molecule into the interphase having a fixed surface density will cause ordering of the alkyl chains (Marqusee and Dill, 1986a; Dill 1987; Dorsey and Dill, 1989), and therefore solute retention is entropically unfavorable. The partition of the solute should be directly proportional to the surface coverage of the alkyl chains until it reaches a maximum where interactions between individual chains become significant. Then the partition coefficient will decrease to zero when the maximum chain density is reached, which is roughly 8 $\mu mol/m^2$. The work by Sentell and Dorsey (1989a) using bonded phases having different bonding densities has confirmed this prediction. Since the end of the alkyl chains on the bonded phase are found to be in rapid motion compared to their attached ends, the solute will prefer to partition to the free ends of the chains. White et al. (1981) observed this type of preferential distribution when they performed neutron scattering experiments on similar bilayer membranes.

The importance of the interphase model is that it predicts the essential of the stationary phase effects on solute retention and selectivity in RPLC. Dependence of solute retention on the surface density of alkyl chains can be explained by the model. Selectivity due to different molecular shapes have been widely noticed (Wise and Sander, 1985; Tanaka et al., 1980; Tanaka et al., 1982; Lochmüller et al., 1985). Using the interphase model, this shape selectivity can be

considered as arising from the fact that more free energy is required to align solutes having shapes parallel to the alkyl chains compared to molecules that have shapes normal to the alkyl chains. Sentell and Dorsey (1989b) measured selectivity of six four-ring polycyclic aromatic hydrocarbons (PAH) on stationary phases having bonding densities from 1.74-4.07 $\mu mol/m^2$. They found that the selectivity of the PAHs increase swith the surface bonding density. The increase in bonding density causes ordering of the alkyl chains and hence elevates the shape selectivity of these PAHs.

In this study, the partition mechanism proposed by Dill and coworkers (Dill 1987; Marquee and Dill, 1986a; Dorsey and Dill, 1989) is tested against an extended data base. Results will be presented in Chapter II to show that the partition mechanism is correct and the solute-solvent free energy of the binary mobile phase and the solutes can be obtained from chromatographic data as predicted from the theory. The ample number of stationary phases for RPLC available today all have distinct retention and selectivity properties. This is likely exerted by the different stationary phase structure and properties on the solute retention mechanism. Many different stationary phases were investigated, and the relative retention strength of them were found to be related to the phase ratio Φ, and the functionality of the silane used in forming the stationary phase. Results of this study will be introduced in Chapter III along with a simple and useful method that was derived to classify these stationary phases according to their retentivity towards all solutes.

CHAPTER II
SOLUTE-SOLVENT INTERACTION FREE ENERGIES
IN REVERSED PHASE LIQUID CHROMATOGRAPHY

Introduction

Binary mobile phases of water and an organic modifier such as acetonitrile, methanol or THF are most commonly used in RPLC. From the partition model proposed by Dill and coworkers (Dill 1987; Marqusee and Dill, 1986a, Dorsey and Dill, 1989), the equilibrium constant, K, of a binary mobile phase with components A and B (a case not discussed in detail in the previous chapter) can be defined as a simple quadratic function of the mobile phase composition and the binary interaction constants between the mobile phase components and the solute

$$\ln K_{C/AB} = (\chi_{SA} - \chi_{SC}) + \phi_B (\chi_{SB} - \chi_{SA} - \chi_{AB}) + \phi_B^2 \chi_{AB} \quad (2\text{-}1)$$

where $0 \leq \phi_B \leq 1$ is the fraction of the mobile phase sites occupied by B molecules, C is the interphase stationary phase, χ_{SA}, χ_{SB}, and χ_{SC} are the binary interaction constants between the solute and the components A (water), B (organic modifier), and C (stationary phase) of the chromatographic system respectively, and χ_{AB} is the binary interaction constant between the mobile phase components A and B.

The quadratic relationship between the equilibrium constant and the volume fraction of organic modifier has been previously reported by

Schoenmakers and his colleagues (Schoenmakers et al., 1978; 1982; 1983) using the solubility parameter theory (Hilderbrand and Scott,1950). Dill and coworkers (Dill 1987; Dorsey and Dill, 1989) have suggested that instead of the quadratic equation, a more useful linear expression can be employed to plot the dependence of retention on mobile phase composition. Since $k'/k_w = K/K_w$ where the subscript w refers to the mobile phase composition when $\phi_B=0$, equation 2-1 can be rearranged to

$$1/\phi_B \ln (k'/k_w) = (\chi_{SB} - \chi_{SA} - \chi_{AB}) + \phi_B \chi_{AB} \qquad (2\text{-}2)$$

As stated in Chapter I, the binary interaction constant χ is often determined by measuring the free energy of transfer or vapor pressure in the Henry's law region (Hill 1986). A careful examination of equation 2-2 reveals that a plot of $1/\phi_B \ln (k'/k_w)$ versus ϕ_B should be linear if all the assumptions made in the partition model hold. Also, the slope of such a plot should give the binary interaction constant χ_{AB}, and the y-intercept at $\phi_B=1$ should be equal to $(\chi_{SB} - \chi_{SA})$. This predicts that the binary interaction constants of mobile phases used in RPLC can be obtained from chromatographic data, and by applying equation 1-7, the solute-solvent free energies between the solute and the two components of the mobile phase in RPLC can be calculated.

In the original derivation of the partition model (Dill 1987), the solute was treated as having a size comparable to the mobile phase components. In actuality, solutes in RPLC are frequently larger than the mobile phase components. Since the cavity term is very important in the partition model, solutes of different sizes should create cavities

of different magnitudes. If the solute molecules are larger than molecules of the mobile phase components, equation 2-2 can be redefined as

$$1/\phi_B \ln (k'/k_w) = n [(\chi_{SB} - \chi_{SA} - \chi_{AB}) + \phi_B \chi_{AB}] \quad (2-3)$$

where n is the ratio of the size of solute to the size of the solvent molecules. This expression results because the number of contacts of solute with the mobile phase components and the size of the cavities are directly proportional to the size of the solute molecule.

Theoretical derivations are not valid until they have been proven experimentally. With this in mind, we tested the prediction of the composition dependence expressed in equations 2-2 and 2-3 with an extensive data base consisting of more than 300 sets of experimental retention data of various solutes on various RPLC columns. We hope to uncover the underlying physical nature of the slope and intercept of this type of composition plot. In agreement with the above predictions, linear dependence of ln k' on surface area or van der Waals volume of solute molecules, and hence the size of the cavity, has been widely observed (Jinno and Kawasaki, 1983a; 1983b; Funasaki et al., 1986; Arai et al., 1987; Kaliszan 1986; Mockel et al., 1987).

Experimental Procedure

We tested the above predictions with a large data base that had been previously used for correlations of solvatochromatic solvent polarity measurements with reversed phase retention (Johnson et al., 1986). The data base is used here in its entirety, with the omission of

six data sets which had retention values at only three mobile phase compositions. Additional data sets generated in our laboratory (Michels 1989) with ethanol-water and propanol-water mobile phases are also included.

Linear regression calculations were performed using the program CURVE FITTER (Interactive Microware, State College, PA, USA) on an Apple II Plus 48K microcomputer. The program was used to calculate the coefficients of correlation, r, and determination, r^2, of the data. This program was also used to extrapolate k' to the composition of pure water, denoted k_w. The ln k_w values are not usually reported in the literature as they are not easily experimentally determined. We extrapolated plots of ln k' versus $E_T(30)$ polarity to the polarity value of 100% water to obtain the ln k_w values used here. This method has been shown to give more reliable values compared to other types of extrapolation (Snyder et al., 1979; Dolan et al., 1979; Schoemakers et al., 1979; Antle et al., 1985; Baty and Sharp, 1988) because the calculated ln k_w for a given solute is consistent among methanol-water, ethanol-water, and acetonitrile-water mobile phases when using this method (Michels 1989). The F-values, which are used to compare the significance level of two variances, were obtained from the StatWorks (Heydon and Son Inc., Philadelphia, PA, USA) program on a Macintosh SE (Apple Computer, Inc., Cupertino, CA, USA) microcomputer. The van der Waals volume of the solutes were calculated using methods reported by Bondi (1964), and the hydrocarbonaceous surface area was obtained from Woodburn (1985).

Results and Discussion

The extensive data base presented here permits certain tests of the cavity contributions to the partition retention model, and the ability of using chromatographic data to calculate binary interaction constants. A typical example of this type of composition plot is shown in Figure 2-1. In the case shown, the solute is 4-nitrophenol and the mobile phase is a series of different acetonitrile-water mixtures. From the figure, it is obvious that this plot provides a linear representation of the experimental data. Table 2-1 presents an extensive compilation of the results of plotting data in this form for a wide range of solutes, stationary phases, and various mobile phase mixtures commonly used in RPLC. From the correlation coefficients shown, it is clear that the degree of linearity of this type of plot is extremely good, with only a very small number of exceptions (Figure 2-2). More than 80% of the data sets are found to have r^2 of 0.9 and higher and almost 50% with r^2 of 0.99 and higher. An F-value, which is used to compare the significance level of fitting the data sets to a quadratic model (Anderson 1987), is calculated and expressed as α in Table 2-1. The results of the F-test reveal that the quadratic model does not fit the data sets significantly better (at the 90% confidence level), except in 148 out of 346 data sets. We conclude that the linear fitting of the data is adequate. The principal exception in the data set is the Sepralyte C-2 column with methanol as modifier. The coefficient of determination, r^2, for this particular data set varies from 0.4460 to 0.9162, with an average value of 0.7287. These poor correlations are probably observed because for the short-chain stationary phases, adsorption is expected to be the dominant retention

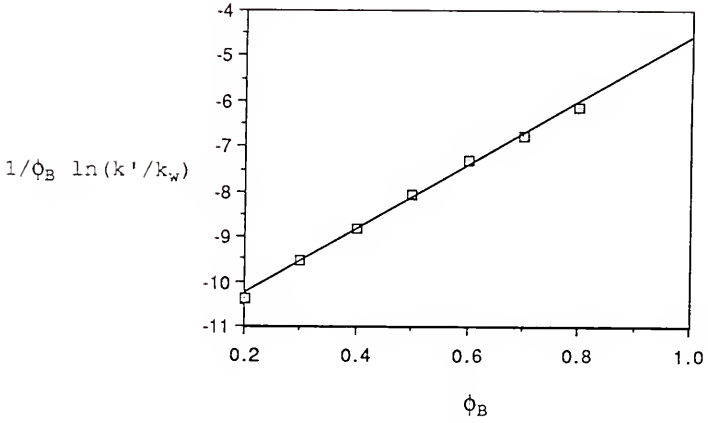


Figure 2-1. Plot of $1/\phi_B \ln(k'/k_w)$ versus ϕ_B for the solute 4-Nitrophenol using Hypersil ODS column and acetonitrile as modifier.

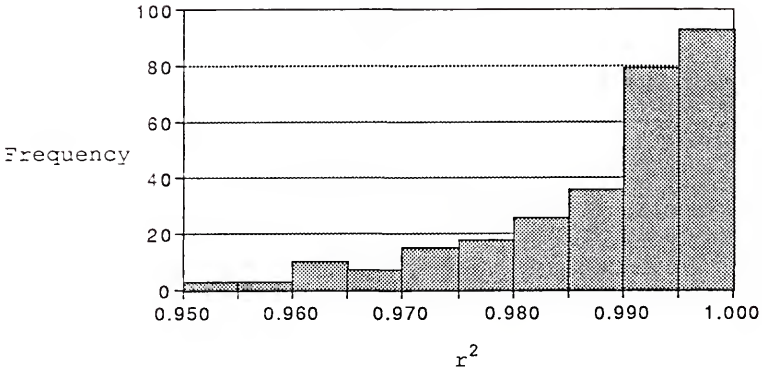


Figure 2-2. Histogram of coefficient of determination, r^2, for the plots of $1/\phi_B \ln(k'/k_w)$ versus ϕ_B for all the data sets.

Table 2-1. Linear regression results of equation 2-3 and results of slopes and intercepts.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ ($\times 10^2$)	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
1	Biphenyl	A	ACN/25-50	45.0	0.9919	13.56	0.9483	0.315	20.62	7.06	4	22.0	Woodburn (1985)
2	Naphthalene	A	"	36.7	0.9865	11.11	0.9230	0.384	16.85	5.74	4	19.7	"
3	Phenanthrene	A	"	48.9	0.9950	14.87	0.9642	0.285	22.53	7.66	4	22.2	"
4	Anthracene	A	"	51.2	0.9917	15.64	0.9385	0.285	23.62	7.98	4	2.53	"
5	Pyrene	A	"	53.9	0.9966	16.51	0.9754	0.260	24.95	8.44	4	55.1	"
6	n-Butylbenzene	A	"	47.5	0.9936	14.36	0.9852	0.315	21.82	7.46	4	48.7	"
7	Benzene	A	"	22.5	0.9522	6.718	0.7748	0.587	10.17	3.45	4	8.22	"
8	Toluene	A	"	28.3	0.9724	8.511	0.8621	0.477	12.89	4.38	4	18.6	"
9	Ethylbenzene	A	"	34.4	0.9815	10.34	0.8987	0.407	15.70	5.36	4	16.6	"
10	n-Propylbenzene	A	"	40.9	0.9873	12.44	0.9255	0.335	18.81	6.37	4	11.4	"
11	p-Xylene	A	"	33.7	0.9752	10.20	0.8737	0.402	15.42	5.22	4	18.8	"
12	o-Xylene	A	"	33.0	0.9789	9.888	0.8866	0.402	15.04	5.15	4	12.1	"
13	m-Diethylbenzene	A	"	46.2	0.9895	14.02	0.9361	0.311	21.24	7.22	4	14.0	"
14	1,2,4-Trimethyl benzene	A	"	38.5	0.9912	11.64	0.9460	0.347	17.68	6.04	4	25.0	"
15	Fluorobenzene	A	"	25.2	0.9630	7.602	0.8226	0.549	11.50	3.90	4	13.9	"
16	Chlorobenzene	A	"	29.3	0.9686	8.928	0.8528	0.490	13.44	4.51	4	48.8	"
17	Bromobenzene	A	"	31.2	0.9803	9.410	0.8969	0.465	14.25	4.84	4	42.6	"
18	Iodobenzene	A	"	34.2	0.9838	10.30	0.9080	0.433	15.63	5.33	4	14.6	"
19	Nitrobenzene	A	"	23.8	0.9642	7.216	0.8256	0.453	10.88	3.66	4	10.7	"
20	Fluoranthene	A	"	54.2	0.9968	16.60	0.9756	0.263	25.09	8.49	4	32.5	"

Column A: 5cmx4.6mm, 10μm Sepralyte C-2(Analytichem); y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

Table 2-1--continued.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ [x10^2]	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.) =(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
21	Biphenyl	B	ACN/30-60	46.8	0.9995	17.27	0.9928	0.315	24.76	7.49	4	24.5	Woodburn (1985)
22	Naphthalene	B	"	39.1	0.9988	14.50	0.9890	0.384	20.76	6.26	4	1160	"
23	Phenanthrene	B	"	50.2	0.9993	18.06	0.9983	0.285	25.99	7.93	4	3.15	"
24	Anthracene	B	"	50.7	0.9994	18.06	0.9983	0.285	26.80	8.16	4	8.20	"
25	Pyrene	B	"	54.0	0.9990	19.77	0.9969	0.260	28.45	8.68	4	2.28	"
26	Chyrsene	B	"	60.7	0.9985	22.31	0.9978	0.227	32.10	9.79	4	1.36	"
27	Fluoranthene	B	"	53.8	0.9994	19.79	0.9960	0.263	28.43	8.64	4	5.66	"
28	n-Butylbenzene	B	"	50.6	0.9994	19.86	0.9906	0.315	28.50	8.64	4	10.5	"
29	Benzene	B	"	26.9	0.9936	10.04	0.9732	0.587	14.30	4.26	4	2860	"
30	Toluene	B	"	32.0	0.9955	11.88	0.9765	0.477	16.95	5.07	4	65.2	"
31	Ethylbenzene	B	"	37.8	0.9977	13.92	0.9828	0.407	19.94	6.02	4	84.5	"
32	n-Propylbenzene	B	"	44.0	0.9989	16.31	0.9888	0.355	23.35	7.04	4	53.4	"
33	p-Xylene	B	"	37.6	0.9983	13.93	0.9878	0.402	19.93	6.00	4	149	"
34	o-Xylene	B	"	36.9	0.9984	13.65	0.9866	0.402	19.55	5.90	4	631	"
35	m-Diethylbenzene	B	"	47.9	0.9998	17.72	0.9921	0.311	25.37	7.65	4	61.7	"
36	1,2,4-Trimethyl benzene	B	"	41.6	0.9993	16.22	0.8665	0.347	23.01	6.79	4	1.24	"
37	Fluorobenzene	B	"	29.0	0.9954	10.73	0.9776	0.549	15.34	4.61	4	4070	"
38	Chlorobenzene	B	"	33.0	0.9964	12.29	0.9797	0.490	17.53	5.24	4	1730	"
39	Bromobenzene	B	"	34.7	0.9973	12.88	0.9837	0.465	18.41	5.53	4	685	"
40	Iodobenzene	B	"	37.1	0.9986	13.78	0.9886	0.433	19.66	5.88	4	545	"
41	Nitrobenzene	B	"	27.1	0.9952	10.05	0.9763	0.453	14.37	4.32	4	76.6	"

Column B: 5cmx4.6mm, 10μm Sepralyte C-4(Analytichem); y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

Table 2-1--continued.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ [x10^2]	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.) =(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
42	Biphenyl	C	ACN/30-80	54.3	0.9985	14.59	0.9873	0.315	24.56	9.97	6	3.93	Woodburn (1985)
43	Naphthalene	C	ACN/40-80	48.5	0.9962	12.59	0.9803	0.384	21.13	8.54	6	6.70	"
44	Phenanthrene	C	"	58.6	0.9990	13.34	0.9949	0.285	24.17	10.83	5	5.37	"
45	Anthracene	C	"	58.9	0.9981	13.47	0.9920	0.285	24.36	10.89	5	3.75	"
46	Pyrene	C	"	61.0	0.9992	13.84	0.9964	0.260	25.12	11.28	5	5.86	"
47	Chyrsene	C	"	67.5	0.9995	15.35	0.9962	0.227	27.83	12.48	5	8.97	"
48	Fluoranthene	C	"	62.1	0.9989	14.13	0.9957	0.263	25.61	11.48	5	4.11	"
49	n-Butylbenzene	C	"	59.8	0.9985	13.64	0.9939	0.315	24.70	11.06	5	3.70	"
50	n-Hexylbenzene	C	"	70.5	0.9988	16.08	0.9949	0.256	29.12	13.04	5	3.67	"
51	Benzene	C	ACN/30-80	34.1	0.9883	9.538	0.9397	0.587	14.53	4.99	5	1.71	"
52	Toluene	C	"	39.7	0.9914	10.96	0.9506	0.477	16.75	5.79	5	1.36	"
53	Ethylbenzene	C	"	45.6	0.9945	12.34	0.9712	0.407	20.68	8.34	6	4.46	"
54	n-Propylbenzene	C	"	51.9	0.9966	13.94	0.9784	0.355	23.44	9.50	6	5.09	"
55	p-Xylene	C	"	45.3	0.9960	12.28	0.9765	0.402	20.58	8.30	6	6.05	"
56	o-Xylene	C	"	44.6	0.9947	12.10	0.9704	0.402	20.24	8.14	6	4.63	"
57	m-Diethylbenzene	C	"	56.0	0.9976	15.03	0.9815	0.311	25.28	10.25	6	7.30	"
58	1,2,4-Trimethyl benzene	C	"	49.5	0.9973	13.31	0.9811	0.347	22.37	9.06	6	6.41	"
59	Fluorobenzene	C	"	36.2	0.9913	9.898	0.9634	0.549	16.52	6.62	6	3.47	"
60	Chlorobenzene	C	"	40.7	0.9951	11.10	0.9734	0.490	18.55	7.45	6	4.29	"
61	Bromobenzene	C	"	42.0	0.9948	11.39	0.9740	0.465	19.07	7.68	6	4.47	"
62	Iodobenzene	C	"	44.5	0.9952	12.25	0.9760	0.433	20.53	8.28	6	6.58	"
63	Nitrobenzene	C	"	35.1	0.9915	9.589	0.9625	0.453	16.00	6.41	6	3.31	"

Column C: 5cmx4.6mm, 10μm Sepralyte C-8(Analytichem); y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

Table 2-1--continued.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ /x10²/	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
64	Biphenyl	D	ACN/50-70	55.1	0.9980	13.46	0.9974	0.315	23.31	9.85	4	12.5	Woodburn (1985)
65	Naphthalene	D	"	49.0	0.9974	11.97	0.9968	0.384	20.73	8.76	4	9.80	"
66	Phenanthrene	D	"	57.6	0.9989	14.06	0.9983	0.285	24.35	10.29	4	17.5	"
67	Anthracene	D	"	58.5	0.9983	14.31	0.9979	0.285	24.76	10.45	4	15.3	"
68	Pyrene	D	"	60.0	0.9986	14.61	0.9983	0.260	25.28	10.67	4	12.7	"
69	Chrysene	D	"	65.9	0.9988	16.13	0.9984	0.227	27.91	11.78	4	6.43	"
70	n-Butylbenzene	D	"	59.0	0.9975	14.43	0.9972	0.315	24.97	10.54	4	62	"
71	n-Hexylbenzene	D	"	69.0	0.9982	16.89	0.9976	0.256	29.22	12.33	4	18.5	"
72	Benzene	D	"	38.2	0.9931	9.305	0.9931	0.587	16.13	6.83	4	181	"
73	Toluene	D	"	43.4	0.9975	10.61	0.9968	0.477	18.36	7.75	4	12.7	"
74	Ethylbenzene	D	"	48.9	0.9976	11.96	0.9973	0.407	20.70	8.74	4	14.0	"
75	n-Propylbenzene	D	"	54.2	0.9980	13.27	0.9978	0.355	22.95	9.68	4	10.3	"
76	p-Xylene	D	"	48.5	0.9979	11.83	0.9974	0.402	20.48	8.65	4	∞	"
77	o-Xylene	D	"	47.8	0.9976	11.63	0.9973	0.402	20.16	8.53	4	∞	"
78	m-Diethylbenzene	D	"	57.7	0.9977	14.10	0.9974	0.311	24.39	10.29	4	18.3	"
79	1,2,4-Trimethyl benzene	D	ACN/40-70	52.2	0.9978	12.73	0.9973	0.347	22.03	9.30	4	1270	"
80	Fluorobenzene	D	"	40.7	0.9963	9.886	0.9961	0.549	17.17	7.28	4	188	"
81	Bromobenzene	D	"	45.9	0.9969	11.09	0.9969	0.465	19.22	8.13	4	188	"
82	Iodobenzene	D	"	47.8	0.9977	11.65	0.9975	0.433	20.19	8.54	4	37	"
83	Nitrobenzene	D	"	39.5	0.9958	9.582	0.9957	0.453	16.63	7.05	4	5440	"

Column D: 5cmx4.6mm, 10μm Sepralyte C-18(Analytichem); y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

Table 2-1--continued.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ [x10^2]	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
84	Acetophenone	E	ACN/50-80	33.7	0.9960	6.721	0.9705	0.398	13.04	6.32	4	31.1	Jandera (1985)
85	Anisole	E	"	40.6	0.9881	8.010	0.9741	0.452	15.65	7.64	4	2.36	"
86	Benzaldehyde	E	"	38.6	0.9947	7.684	0.9687	0.465	14.93	7.25	4	17.0	"
87	Benzonitrile	E	"	39.2	0.9920	7.784	0.9728	0.469	15.15	7.37	4	4.41	"
88	Benzophenone	E	"	47.9	0.9976	9.480	0.9851	0.274	18.51	9.03	4	170	"
89	Benzotrichloride	E	"	52.4	0.9989	10.36	0.9881	0.330	20.21	9.85	4	20.1	"
90	Bromobenzene	E	"	44.4	0.9942	8.750	0.9804	0.465	17.10	8.35	4	6.26	"
91	n-Butylbromide	E	"	44.8	0.9966	8.870	0.9882	0.483	17.30	8.43	4	58.3	"
92	n-Butylphenyl carbamate	E	"	48.0	0.9981	9.510	0.9810	0.251	18.52	9.01	4	218	"
93	m-Cresol	E	"	39.4	0.9833	7.777	0.9706	0.420	15.20	7.42	4	1.59	"
94	o-Cresol	E	"	39.0	0.9959	7.785	0.9625	0.420	15.09	7.31	4	98.2	"
95	p-Cresol	E	"	41.9	0.9980	8.380	0.9716	0.420	16.24	7.86	4	512	"
96	Ethyl benzoate	E	"	45.4	0.9934	9.000	0.9763	0.333	17.53	8.53	4	6.13	"
97	Methyl benzoate	E	"	39.0	0.9926	7.771	0.9646	0.379	15.09	7.32	4	9.22	"
98	Nitrobenzene	E	"	40.0	0.9948	7.933	0.9809	0.453	15.46	7.53	4	7.29	"
99	Phenetole	E	"	41.9	0.9765	8.263	0.9632	0.389	16.15	7.89	4	1.25	"
100	Phenyl acetate	E	"	36.0	0.9911	7.188	0.9624	0.381	13.93	6.74	4	7.10	"
101	n-Propylphenyl ether	E	"	46.1	0.9960	9.160	0.9741	0.341	17.81	8.65	4	23.2	"
102	Chlorobenzene	E	"	44.6	0.9969	8.810	0.9831	0.490	17.21	8.40	4	23.7	"
103	Chlorobromuron	E	"	46.6	0.9966	9.230	0.9832	0.231	18.00	8.77	4	16.5	"
104	Di-n-butyl ether	E	"	46.6	0.9947	9.200	0.9922	0.307	17.98	8.78	4	32.8	"
105	n-Heptane	E	"	54.5	0.9986	10.78	0.9874	0.361	21.03	10.25	4	36.9	"
106	Linuron	E	"	46.6	0.9970	9.250	0.9823	0.237	18.02	8.77	4	34.9	"
107	n-Octane	E	"	59.0	0.9976	11.64	0.9907	0.320	22.74	11.10	4	45.1	"
108	Styrene	E	"	45.5	0.9944	8.970	0.9865	0.428	17.54	8.57	4	6.52	"

Column E: 30cmx3.8mm, 7.5μm Silasorb C-8(Lachema); y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

Table 2-1--continued.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ [x10^2]	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
109	4-Nitrophenol	F	ACN/20-80	27.3	0.9936	7.060	0.9963	0.416	11.67	4.61	7	4.48	Hanai and Hubert (1983)
110	2,4-Dinitrophenol	F	"	31.8	0.9917	8.371	0.9827	0.344	13.68	5.31	7	3.66	"
111	3-Bromophenol	F	"	34.7	0.9919	9.020	0.9971	0.430	14.89	5.87	7	2.38	"
112	4-Chloro-3-methyl phenol	F	ACN/30-80	37.9	0.9847	9.593	0.9962	0.381	16.09	6.50	6	1.87	"
113	2,5-Dichlorophenol	F	"	40.6	0.9877	10.37	0.9939	0.389	17.32	6.95	6	4.36	"
114	4-Chloro-3,5-dimethylphenol	F	"	42.3	0.9434	10.45	0.9668	0.331	17.81	7.36	6	0.864	"
115	2,4,5-Trichloro phenol	F	ACN/40-80	45.7	0.9688	10.41	0.9963	0.345	18.61	8.20	5	0.853	"
116	2,3,4,5-Tetrachloro phenol	F	"	52.4	0.9702	11.99	0.9965	0.309	21.39	9.40	5	0.907	"
117	Aniline	F	ACN/10-70	19.5	0.9951	14.13	0.8594	0.503	14.64	0.51	7	4.97	"
118	N-Methylaniline	F	ACN/20-70	28.0	0.9901	8.239	0.9606	0.420	12.43	4.19	6	5.16	"
119	N-Ethylaniline	F	"	33.0	0.9904	9.794	0.9608	0.365	14.71	4.92	6	5.09	"
120	N-Butylaniline	F	ACN/40-70	53.9	0.9973	14.44	0.9957	0.289	23.07	8.63	4	74.0	"
121	N,N-Dimethylaniline	F	ACN/30-70	38.4	0.9981	11.22	0.9850	0.366	17.05	5.83	5	8.13	"
122	N,N-Diethylaniline	F	ACN/40-70	54.1	0.9970	14.44	0.9961	0.290	23.09	8.65	4	67.3	"
123	2-Methylaniline	F	ACN/10-70	24.5	0.9964	6.830	0.9906	0.420	10.68	3.85	7	41.0	"
124	3-Methylaniline	F	"	25.3	0.9967	7.156	0.9929	0.420	11.11	3.95	7	6.97	"
125	4-Methylaniline	F	"	25.4	0.9973	6.916	0.9965	0.420	10.98	4.06	7	7.15	"

Column F: 15cmx4.1mm, 5μm Hypersil ODS(Shandon Southern); y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

Table 2-1--continued.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ /x10^2/	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.) =(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
126	2,4-Dimethylaniline	F	ACN/20-70	31.4	0.9978	9.069	0.9921	0.361	13.84	4.77	6	23.8	Hanai and Hubert (1983)
127	4-Methoxyaniline	F	ACN/10-70	21.3	0.9981	5.275	0.9334	0.401	8.924	3.65	7	1.28	"
128	2,4-Diethoxyaniline	F	ACN/20-70	36.5	0.9983	10.50	0.9947	0.269	16.07	5.57	6	64.3	"
129	2-Chloroaniline	F	"	30.7	0.9927	8.931	0.9713	0.431	13.55	4.62	6	4.56	"
130	3-Chloroaniline	F	"	32.1	0.9949	9.406	0.9799	0.431	14.25	4.84	6	5.20	"
131	4-Chloroaniline	F	"	31.3	0.9964	9.080	0.9877	0.431	13.83	4.75	6	6.12	"
132	2,5-Dichloroaniline	F	ACN/30-70	44.3	0.9984	12.75	0.9916	0.377	19.49	6.74	5	14.7	"
133	3,4-Dichloroaniline	F	ACN/30-70	43.0	0.9982	12.46	0.9945	0.377	19.00	6.54	5	31.6	"
134	4-Bromoaniline	F	ACN/20-70	33.5	0.9936	9.755	0.9879	0.414	14.84	5.09	6	7.57	"
135	2-Nitroaniline	F	ACN/10-70	28.6	0.9936	8.080	0.9706	0.402	12.50	4.42	7	3.09	"
136	3-Nitroaniline	F	"	26.2	0.9895	7.808	0.9292	0.402	11.69	3.88	7	2.74	"
137	4-Nitroaniline	F	"	26.3	0.9924	7.445	0.9597	0.402	11.47	4.03	7	3.26	"
138	Pyridine	F	"	17.0	0.9883	5.945	0.9990	0.636	8.102	2.16	6	1.75	"
139	2-Aminopyridine	F	"	12.5	0.9017	5.317	0.9782	0.539	6.472	1.16	6	7.64	"
140	3-Aminopyridine	F	"	15.6	0.9661	5.942	0.9974	0.539	7.675	1.73	6	1.14	"
141	2-Methylpyridine	F	"	22.7	0.9887	8.053	0.9976	0.509	10.87	2.82	6	5.15	"
142	3-Methylpyridine	F	"	23.5	0.9940	7.864	0.9979	0.509	10.99	3.13	6	2.95	"
143	4-Methylpyridine	F	"	23.6	0.9920	7.930	0.9977	0.509	11.02	3.09	6	1.13	"
144	4-Ethylpyridine	F	ACN/20-70	28.2	0.9973	7.955	0.9915	0.430	12.35	4.40	6	1.00	"
145	4-tert-Butylpyridine	F	ACN/30-70	37.7	0.9956	10.84	0.9985	0.328	16.62	5.78	5	1.17	"
146	2,4-Dimethylpyridine	F	"	26.8	0.9967	7.681	0.9967	0.424	11.77	4.09	5	1.39	"

Column F: 15cmx4.1mm, 5µm Hypersil ODS(Shandon Southern); y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

Table 2-1--continued.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ $(x10^2)$	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
147	2,6-Dimethylpyridine	F	ACN/10-70	18.1	0.9920	6.979	0.9904	0.424	10.87	3.89	6	1.37	Hanai and Hubert (1983)
148	2,5-Dimethylpyridine	F	ACN/20-70	26.6	0.9973	7.520	0.9919	0.424	11.66	4.14	6	0.0985	"
149	Pyrazine	F	ACN/10-70	10.2	0.9196	4.308	0.9866	0.694	5.251	0.943	6	3.44	"
150	2-Methylpyrazine	F	"	14.2	0.9433	5.931	0.9979	0.545	7.292	1.36	6	1.52	"
151	2,5-Dimethylpyrazine	F	"	18.2	0.9530	7.548	0.9969	0.449	9.310	1.76	6	0.760	"
152	2,6-Dimethylpyrazine	F	"	18.1	0.9472	7.396	0.9963	0.449	9.183	1.79	6	0.916	"
153	Quinoline	F	ACN/20-70	29.8	0.9959	8.302	0.9899	0.404	12.96	4.66	6	1.30	"
154	2-Methylquinoline	F	"	33.6	0.9971	9.465	0.9923	0.349	14.70	5.24	6	1.06	"
155	4-Methylquinoline	F	"	32.4	0.9964	9.221	0.9975	0.349	14.20	4.98	6	1.20	"
156	8-Methylquinoline	F	"	34.7	0.9985	9.799	0.9989	0.349	15.16	5.36	6	0.764	"
157	5-Aminoindan	F	"	34.0	0.9981	9.701	0.9962	0.353	14.92	5.22	6	51.7	"
158	5-Aminoindole	F	ACN/10-70	21.0	0.9968	5.515	0.9678	0.372	9.003	3.49	7	1.29	"
159	1-Aminonaphthalene	F	ACN/20-70	36.1	0.9976	10.37	0.9920	0.346	15.87	5.50	6	17.2	"
160	2-Aminonaphthalene	F	ACN/20-70	37.1	0.9977	10.65	0.9941	0.346	16.31	5.66	6	13.9	"
161	1-Aminoanthracene	F	ACN/30-70	53.3	0.9976	15.75	0.9845	0.264	24.00	8.25	5	1.57	"
162	1-Aminopyrene	F	ACN/40-70	55.5	0.9937	14.80	0.9986	0.242	23.70	8.90	4	∞	"

Column F: 15cmx4.1mm, 5μm Hypersil ODS(Shandon Southern); y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

Table 2-1--continued.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ /x10^2/	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
163	Phenol	G	ACN/10-90	20.9	0.9975	5.234	0.9934	0.403	8.937	3.70	9	1.24	Hanai and Hubert (1983)
164	2-Methylphenol	G	ACN/20-90	27.7	0.9962	6.993	0.9905	0.436	11.84	4.85	8	40.9	"
165	3-Methylphenol	G	"	27.3	0.9955	6.860	0.9941	0.436	11.64	4.78	8	10.8	"
166	4-Methylphenol	G	ACN/10-90	27.1	0.9976	6.542	0.9870	0.436	11.44	4.90	8	4.18	"
167	2,3-Dimethylphenol	G	ACN/30-90	33.2	0.9902	8.019	0.9930	0.372	13.92	5.90	7	50.6	"
168	2,4-Dimethylphenol	G	"	32.9	0.9842	7.928	0.9935	0.372	13.82	5.89	7	7.63	"
169	2,5-Dimethylphenol	G	"	33.8	0.9908	8.205	0.9925	0.372	14.21	6.01	7	47.2	"
170	2,6-Dimethylphenol	G	"	33.5	0.9921	8.040	0.9903	0.372	13.99	5.95	7	35.2	"
171	3,4-Dimethylphenol	G	ACN/20-90	32.2	0.9939	7.947	0.9962	0.372	13.59	5.64	8	2.18	"
172	3,5-Dimethylphenol	G	"	33.2	0.9944	8.212	0.9957	0.372	14.03	5.82	8	4.28	"
173	2,3,5-Trimethyl phenol	G	ACN/30-90	38.9	0.9903	9.366	0.9927	0.325	16.28	6.91	7	27.5	"
174	2,3,6-Trimethyl phenol	G	"	38.5	0.9921	9.286	0.9897	0.325	16.15	6.86	7	71.4	"
175	2,4,6-Trimethyl phenol	G	"	39.0	0.9922	9.412	0.9903	0.325	16.37	6.96	7	53.8	"
176	2,3,5,6-Tetramethyl phenol	G	ACN/40-90	37.3	0.9894	8.013	0.9892	0.281	14.95	6.94	6	15.3	"
177	2-Ethylphenol	G	ACN/20-90	34.2	0.9943	8.512	0.9956	0.377	14.50	5.99	8	3.19	"
178	3-Ethylphenol	G	"	33.9	0.9949	8.452	0.9950	0.377	14.38	5.93	8	8.62	"
179	4-Ethylphenol	G	"	34.1	0.9946	8.533	0.9954	0.377	14.50	5.97	8	6.19	"
180	2-Chlorophenol	G	"	29.3	0.9949	7.307	0.9949	0.448	12.43	5.12	8	23.6	"
181	3-Chlorophenol	G	"	32.4	0.9946	8.037	0.9948	0.448	13.71	5.67	8	11.0	"
182	4-Chlorophenol	G	"	32.0	0.9949	8.087	0.9955	0.448	13.68	5.59	8	5.18	"
183	2,3-Dichlorophenol	G	ACN/30-90	38.0	0.9876	9.207	0.9948	0.389	15.94	6.73	7	3.04	"

Column G: 15cmx4.1mm, 5µm Unisil Q C-18(Gasukuro Kogyo); y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

Table 2-1--continued.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ ($x10^2$)	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
184	2,4-Dichlorophenol	G	ACN/30-90	39.3	0.9891	9.517	0.9942	0.389	16.50	6.89	7	7.86	Hanai and Hubert (1983)
185	2,5-Dichlorophenol	G	"	39.6	0.9902	9.602	0.9931	0.389	16.61	7.03	7	44.4	"
186	2,6-Dichlorophenol	G	"	36.0	0.9918	8.734	0.9912	0.389	15.15	6.42	7	189	"
187	3,4-Dichlorophenol	G	"	40.6	0.9885	9.844	0.9948	0.389	17.05	7.21	7	6.49	"
188	3,5-Dichlorophenol	G	"	43.4	0.9904	10.48	0.9935	0.389	18.18	7.70	7	20.0	"
189	2,3,4-Trichloro phenol	G	ACN/40-90	43.1	0.9813	9.453	0.9964	0.345	17.35	7.90	6	4.63	"
190	2,3,5-Trichloro phenol	G	ACN/30-90	38.8	0.9915	9.356	0.9926	0.345	16.24	6.88	7	33.0	"
191	2,3,6-Trichloro phenol	G	ACN/40-90	41.8	0.9844	9.114	0.9949	0.345	16.82	7.71	6	16.7	"
192	2,4,5-Trichloro phenol	G	"	44.8	0.9830	9.759	0.9954	0.345	17.99	8.23	6	12.8	"
193	2,4,6-Trichloro phenol	G	"	43.0	0.9855	9.347	0.9941	0.345	17.29	7.94	6	22.5	"
194	3,4,5-Trichloro phenol	G	"	45.9	0.9821	10.04	0.9955	0.345	18.49	8.45	6	6.57	"
195	2,3,4,5-Tetrachloro phenol	G	"	50.9	0.9835	11.10	0.9948	0.309	20.48	9.38	6	10.0	"
196	2,3,5,6-Tetrachloro phenol	G	"	49.6	0.9852	11.29	0.9930	0.309	20.57	9.28	6	26.1	"
197	Pentachlorophenol	G	ACN/50-90	48.6	0.9916	8.990	0.9955	0.280	18.37	9.38	5	10.9	"
198	2-Chloro-5-methyl phenol	G	ACN/30-90	35.5	0.9905	8.632	0.9933	0.381	14.93	6.30	7	55.8	"
199	4-Chloro-2-methyl phenol	G	"	38.6	0.9898	9.301	0.9942	0.381	16.14	6.84	7	18.6	"
200	4-Chloro-3-methyl phenol	G	"	37.0	0.9881	8.961	0.9950	0.381	15.52	6.56	7	10.2	"

Column G: 15cmx4.1mm, 5μm Unisil Q C-18(Gasukuro Kogyo); y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

Table 2-1--continued.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ ($\times 10^2$)	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
201	2-Bromophenol	G	ACN/20-90	31.1	0.9952	7.771	0.9948	0.430	13.22	5.45	8	16.7	Hanai and Hubert (1983)
202	3-Bromophenol	G	"	34.2	0.9949	8.443	0.9954	0.430	14.42	5.98	8	7.79	"
203	4-Bromophenol	G	"	34.0	0.9942	8.439	0.9963	0.430	14.38	5.94	8	3.05	"
204	2,4-Dibromophenol	G	ACN/30-90	43.2	0.9892	10.48	0.9941	0.364	18.15	7.67	7	10.3	"
205	2,6-Dibromophenol	G	"	39.5	0.9919	9.546	0.9909	0.364	16.58	7.03	7	155	"
206	2-Nitrophenol	G	ACN/20-90	29.2	0.9950	7.280	0.9784	0.416	12.41	5.13	8	7.99	"
207	3-Nitrophenol	G	"	27.7	0.9943	6.877	0.9962	0.416	11.73	4.85	8	6.30	"
208	4-Nitrophenol	G	ACN/10-90	26.9	0.9965	6.571	0.9969	0.416	11.34	4.77	9	0.877	"
209	2,4-Dinitrophenol	G	ACN/20-90	31.1	0.9942	7.787	0.9877	0.344	13.23	5.44	8	9.96	"
210	2,5-Dinitrophenol	G	"	31.8	0.9933	7.996	0.9794	0.344	13.58	5.58	8	5.28	"
211	2,6-Dinitrophenol	G	"	29.4	0.9918	7.376	0.9660	0.344	12.52	5.14	8	3.72	"
212	3,4-Dinitrophenol	G	"	36.3	0.9921	9.148	0.9982	0.344	15.45	6.30	8	4.86	"
213	2-Hydroxyaceto phenone	G	ACN/10-90	19.6	0.9821	5.968	0.9920	0.370	9.035	3.07	9	1.25	"
214	4-tert-Butylphenol	G	ACN/40-90	41.6	0.9800	9.071	0.9962	0.296	16.71	7.64	6	5.96	"
215	4-Hydroxypropyl benzoate	G	ACN/30-90	37.9	0.9826	9.187	0.9968	0.281	15.88	6.69	7	1.65	"
216	4-Hydroxybutyl benzoate	G	"	45.6	0.9849	11.06	0.9961	0.255	19.13	8.07	7	2.47	"
217	4-Chloro-3,5-dimethylphenol	G	"	42.4	0.9884	10.24	0.9947	0.331	17.76	7.52	7	6.31	"
218	1,3-Dihydroxybenzene	G	ACN/10-90	14.0	0.9879	4.156	0.9896	0.478	6.379	2.22	9	1.17	"
219	1,2-Dihydroxybenzene	G	"	15.9	0.9927	4.461	0.9931	0.478	7.066	2.61	9	1.16	"
220	1,4-Dihydroxybenzene	G	"	10.1	0.9903	3.079	0.9901	0.478	4.580	1.50	9	1.57	"
221	2-Hydroxynaphthalene	G	ACN/30-90	36.2	0.9849	8.786	0.9963	0.357	15.19	6.40	7	4.85	"
222	1-Hydroxynaphthalene	G	"	38.3	0.9877	9.302	0.9952	0.357	16.09	6.79	7	11.0	"
223	1-Hydroxy-2,4-dinitro naphthalene	G	ACN/40-90	44.5	0.9850	9.697	0.9942	0.263	17.90	8.20	6	23.8	"

Column G: 15cmx4.1mm, 5μm Unisil Q C-18(Gasukuro Kogyo); y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

Table 2-1--continued.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ (x10^2)	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
224	Anthracene	C	MeOH/50-80	82.4	0.9996	7.510	0.9984	0.218	21.76	14.25	4	46.7	Woodburn (1985)
225	Pyrene	C	"	87.3	0.9999	7.980	0.9995	0.199	23.10	15.12	4	0.700	"
226	n-Butylbenzene	C	"	82.6	0.9992	7.500	0.9962	0.241	21.78	14.28	4	10.4	"
227	Benzene	C	"	47.2	0.9955	4.303	0.9761	0.449	12.43	8.13	4	37.7	"
228	Toluene	C	"	54.1	0.9841	5.090	0.9839	0.365	14.66	9.57	4	233	"
229	Ethylbenzene	C	"	64.0	0.9971	5.830	0.9842	0.311	16.87	11.04	4	2720	"
230	n-Propylbenzene	C	"	73.3	0.9983	6.690	0.9919	0.271	19.35	12.66	4	202	"
231	p-Xylene	C	"	62.8	0.9972	5.760	0.9843	0.307	16.59	10.83	4	18.9	"
232	o-Xylene	C	"	59.7	0.9861	5.600	0.9876	0.307	16.15	10.55	4	∞	"
233	m-Diethylbenzene	C	"	79.5	0.9986	7.260	0.9936	0.238	20.99	13.73	4	425	"
234	1,2,4-Trimethyl benzene	C	"	69.0	0.9981	6.090	0.9969	0.265	17.69	11.60	4	1.08	"
235	Fluorobenzene	C	"	50.8	0.9955	4.606	0.9767	0.420	13.36	8.75	4	630	"
236	Chlorobenzene	C	"	57.1	0.9968	5.200	0.9832	0.375	15.06	9.86	4	23.0	"
237	Bromobenzene	C	"	59.1	0.9979	5.380	0.9883	0.356	15.58	10.20	4	42.5	"
238	Iodobenzene	C	"	62.5	0.9978	5.700	0.9877	0.332	16.49	10.79	4	20.0	"
239	Nitrobenzene	C	"	47.2	0.9963	4.276	0.9812	0.347	12.41	8.13	4	87.0	"

Column C: 5cmx4.6mm, 10μm Sepralyte C-8(Analytichem); y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

Table 2-1--continued.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ ($\times 10^2$)	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
240	Biphenyl	B	MeOH/50-75	68.7	0.9887	6.108	0.9903	0.241	18.37	12.26	4	67	Woodburn (1985)
241	Naphthalene	B	MeOH/40-75	54.7	0.9897	4.267	0.9596	0.294	14.04	9.77	5	30.3	"
242	Phenanthrene	B	MeOH/50-75	73.9	0.9897	6.532	0.9958	0.218	19.66	13.13	4	2.75	"
243	Anthracene	B	"	75.3	0.9898	6.739	0.9926	0.218	20.20	13.46	4	20.7	"
244	Pyrene	B	"	80.3	0.9906	7.125	0.9970	0.199	21.41	14.29	4	1.93	"
245	Chrysene	B	"	89.8	0.9913	7.898	0.9983	0.173	23.84	15.94	4	1.90	"
246	n-Butylbenzene	B	"	77.8	0.9990	6.647	0.9938	0.241	20.05	13.40	4	12.5	"
247	Benzene	B	MeOH/40-75	39.5	0.9853	2.998	0.8461	0.449	9.722	6.724	5	8.43	"
248	Toluene	B	"	46.7	0.9910	3.490	0.9019	0.365	11.45	7.96	5	7.87	"
249	Ethylbenzene	B	"	54.8	0.9924	4.045	0.9423	0.311	13.41	9.37	5	10.1	"
250	n-Propylbenzene	B	MeOH/50-75	68.4	0.9983	5.844	0.9893	0.271	17.60	11.76	4	13.6	"
251	p-Xylene	B	MeOH/40-75	54.2	0.9933	4.005	0.9286	0.307	13.26	9.26	4	7.17	"
252	o-Xylene	B	MeOH/50-75	61.2	0.9976	4.820	0.9867	0.307	14.54	9.72	4	5.18	"
253	m-Diethylbenzene	B	"	74.8	0.9976	6.414	0.9832	0.238	19.26	12.85	4	3.91	"
254	1,2,4-Trimethyl benzene	B	MeOH/40-75	60.6	0.9958	4.510	0.9605	0.265	14.89	10.38	5	14.3	"
255	Fluorobenzene	B	"	43.0	0.9872	3.185	0.8675	0.420	10.49	7.31	5	11.4	"
256	Chlorobenzene	B	"	48.9	0.9913	3.656	0.9091	0.375	12.01	8.35	5	9.61	"
257	Bromobenzene	B	"	51.0	0.9905	3.824	0.9037	0.356	12.54	8.72	5	16.4	"
258	Iodobenzene	B	"	55.0	0.9950	4.154	0.9513	0.332	13.58	9.43	5	12.7	"
259	Nitrobenzene	B	"	41.8	0.9903	3.181	0.8995	0.347	10.32	7.14	5	7.54	"

Column B: 5cmx4.6mm, 10µm Sepralyte C-4(Analytichem); y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

Table 2-1--continued.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ ($x10^2$)	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
260	Biphenyl	A	MeOH/35-60	61.6	0.9895	4.746	0.8279	0.241	15.58		4	∞	Woodburn (1985)
261	Naphthalene	A	"	51.9	0.9823	4.088	0.7406	0.294	13.17		4	540	"
262	Phenanthrene	A	"	69.2	0.9926	5.288	0.8773	0.218	17.48		4	721	"
263	Anthracene	A	"	70.7	0.9923	5.414	0.8703	0.218	17.88		4	134	"
264	Pyrene	A	"	77.8	0.9945	5.841	0.9054	0.199	19.58		4	329	"
265	Fluoranthene	A	"	74.8	0.9983	5.824	0.9162	0.201	19.58		4	1900	"
266	n-Butylbenzene	A	"	65.1	0.9895	5.078	0.8293	0.241	16.51		4	978	"
267	Benzene	A	"	33.1	0.9535	2.857	0.5305	0.449	8.583		4	666	"
268	Toluene	A	"	39.2	0.9672	3.287	0.6115	0.365	10.11		4	1270	"
269	Ethylbenzene	A	"	46.1	0.9763	3.717	0.6796	0.311	11.77		4	13100	"
270	n-Propylbenzene	A	"	54.6	0.9837	4.254	0.7513	0.271	13.83		4	28300	"
271	p-Xylene	A	"	47.0	0.9756	3.858	0.6584	0.307	12.06		4	15.8	"
272	o-Xylene	A	"	45.4	0.9765	3.745	0.6932	0.307	11.68		4	381	"
273	m-Diethylbenzene	A	"	60.7	0.9899	4.593	0.8411	0.238	15.28		4	23.7	"
274	1,2,4-Trimethyl benzene	A	"	53.3	0.9852	4.231	0.7819	0.265	13.58		4	230	"
275	Fluorobenzene	A	"	36.1	0.9633	3.017	0.5809	0.420	9.286		4	605	"
276	Chlorobenzene	A	"	42.2	0.9723	3.487	0.6506	0.375	10.84		4	8420	"
277	Bromobenzene	A	"	45.1	0.9866	3.745	0.6580	0.356	11.61		4	448	"
278	Iodobenzene	A	"	48.3	0.9802	3.820	0.7038	0.332	12.27		4	377	"
279	Nitrobenzene	A	"	36.2	0.9629	3.076	0.4660	0.347	9.016		4	11.2	"

Column A: 5cmx4.6mm, 10μm Sepralyte C-2(Analytichem); y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

Table 2-1--continued.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ ($\times 10^2$)	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.) =(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
280	p-Nitrophenol	H	EtOH/30-70	26.0	0.9948	7.841	0.9554	0.469	13.15	5.31	5	2.46	Michels (1989)
281	p-Nitroanisole	H	"	34.7	0.9960	10.16	0.9923	0.415	17.39	7.23	5	0.673	"
282	Benzophenone	H	EtOH/40-80	39.4	0.9924	9.920	0.9990	0.309	18.80	8.88	5	0.738	"
283	Naphthalene	H	"	42.3	0.9965	10.76	0.9954	0.432	20.27	9.51	5	7.33	"
284	Nitrobenzene	H	EtOH/30-70	28.7	0.9945	8.584	0.9840	0.510	14.52	5.94	5	6.65	"
285	Benzylamine	H	"	24.0	0.9954	7.034	0.9948	0.480	12.02	4.99	5	3.54	"
286	Toluene	H	EtOH/40-80	38.1	0.9958	9.230	0.9923	0.537	17.70	8.47	5	27.5	"
287	Ethylbenzene	H	"	44.2	0.9955	11.27	0.9931	0.458	21.20	9.93	5	10.4	"
288	n-Propylbenzene	H	EtOH/50-90	50.9	0.9948	10.96	0.9899	0.399	23.14	12.2	5	14.6	"
289	n-Butylbenzene	H	"	55.9	0.9932	12.02	0.9927	0.354	25.42	13.4	5	34.9	"
290	p-Nitrophenol	H	PrOH/30-50	40.3	0.9851	12.49	0.9952	0.619	15.10	2.61	5	0.660	"
291	p-Nitroanisole	H	PrOH/30-70	41.8	0.9955	4.501	0.9823	0.548	8.779	4.28	7	0.837	"
292	Benzophenone	H	"	54.6	0.9953	6.945	0.9881	0.408	12.52	5.58	6	0.778	"
293	Naphthalene	H	"	59.8	0.9961	8.493	0.9860	0.570	14.74	6.25	5	0.698	"
294	Nitrobenzene	H	"	41.3	0.9957	4.105	0.9652	0.673	8.341	4.24	7	0.829	"
295	Benzylamine	H	PrOH/30-50	29.8	0.9774	5.932	0.9995	0.633	8.566	2.63	5	0.500	"
296	Toluene	H	PrOH/30-70	54.3	0.9973	6.859	0.9819	0.709	12.55	5.69	5	0.697	"
297	Ethylbenzene	H	"	59.8	0.9961	8.493	0.9860	0.605	14.74	6.25	5	0.698	"
298	n-Propylbenzene	H	"	66.7	0.9957	9.970	0.9863	0.527	16.93	6.96	5	0.692	"
299	n-Butylbenzene	H	"	72.8	0.9949	11.45	0.9884	0.468	19.03	7.58	5	0.694	"
300	2-Nitroaniline	H	ACN/10-60	23.4	0.9909	7.945	0.8806	0.402	11.40	3.46	6	19.3	Johnson (1986)
301	4-Nitroaniline	H	"	20.8	0.9903	7.255	0.9917	0.402	9.559	2.30	6	3.89	"
302	4-Nitroanisole	H	ACN/10-80	31.1	0.9859	8.963	0.8981	0.368	13.84	4.88	8	7.51	"
303	4-Nitrophenol	H	ACN/10-80	24.5	0.9948	6.857	0.9898	0.416	10.69	3.83	6	1.16	"

Column H: 15cmx4.6mm, 5μm Ultrasphere ODS(Altex); y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

Table 2-1--continued.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ ($\times 10^2$)	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
304	Phenol	E	ACN/50-80	43.4	0.9691	8.810	0.9169	0.403	16.87	8.06	4	4.06	Jandera (1985)
305	Acetophenone	E	MeOH/60-90	50.5	0.9989	5.488	0.9940	0.305	14.25	8.76	4	9.00	"
306	Anisole	E	"	57.0	0.9989	6.210	0.9673	0.346	16.08	9.87	4	722	"
307	Benzaldehyde	E	"	55.1	0.9777	6.070	0.9393	0.356	15.61	9.54	4	11900	"
308	Benzonitrile	E	"	53.6	0.9952	5.850	0.9817	0.359	15.14	9.29	4	14.5	"
309	Benzophenone	E	"	69.4	0.9980	7.650	0.9907	0.210	19.70	12.1	4	137	"
310	Benzotrichloride	E	"	79.8	0.9988	8.710	0.9934	0.253	22.56	13.9	4	15.5	"
311	Bromobenzene	E	"	67.1	0.9951	7.320	0.9822	0.356	18.94	11.6	4	242	"
312	n-Butyl bromide	E	"	66.9	0.9927	7.320	0.9753	0.369	18.92	11.6	4	170	"
313	n-Butylphenyl carbamate	E	"	75.3	0.9926	8.270	0.9771	0.192	21.34	13.1	4	9.50	"
314	Chlorobenzene	E	"	64.7	0.9944	7.090	0.9802	0.375	18.31	11.2	4	562	"
315	Chlorobromuron	E	"	73.9	0.9922	8.070	0.9725	0.177	20.87	12.8	4	9.56	"
316	m-Cresol	E	"	62.6	0.9772	4.725	0.9688	0.321	12.21	7.49	4	0.978	"
317	o-Cresol	E	"	61.0	0.9792	6.740	0.9425	0.321	17.31	10.6	4	7.86	"
318	p-Cresol	E	"	58.8	0.9848	5.028	0.9722	0.321	12.94	7.91	4	45.0	"
319	Di-n-butyl ether	E	"	74.5	0.9990	8.070	0.9929	0.235	20.97	12.9	4	5.27	"
320	Ethyl benzoate	E	"	68.2	0.9978	7.380	0.9917	0.255	19.20	11.8	4	56.5	"
321	n-Heptane	E	"	95.1	0.9962	10.36	0.9865	0.277	26.83	16.5	4	1830	"
322	Linuron	E	"	70.1	0.9985	7.640	0.9933	0.181	19.80	12.2	4	490	"
323	Methyl benzoate	E	"	59.4	0.9981	6.510	0.9914	0.290	16.82	10.3	4	202	"
324	Nitrobenzene	E	"	58.2	0.9841	6.370	0.9531	0.347	16.43	10.1	4	83.2	"
325	Phenetole	E	"	61.3	0.9971	6.710	0.9856	0.298	17.33	10.6	4	3.02	"
326	Phenol	E	"	61.4	0.9198	6.860	0.8271	0.403	17.47	10.6	4	154	"
327	Phenyl acetate	E	"	50.1	0.9985	5.509	0.9919	0.292	14.21	8.70	4	31	"
328	n-Propylphenyl ether	E	"	68.0	0.9957	7.400	0.9841	0.261	19.19	11.8	4	∞	"
329	Styrene	E	"	65.7	0.9961	7.170	0.9854	0.328	18.56	11.4	4	3800	"

Column E: 30cmx3.8mm, 7.5µm Silasorb C-8(Lachema); y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

Table 2-1--continued.

Data	Solute	Column	Solvent/% Range	log k'vs $E_T(30)$ ($x10^2$)	r^2	Slope of equation 2-3	r^2	1/n	-(y-int.)	-(y-int.) at ϕ_B=1	N	α	Reference
330	Benzene	H	ACN/31.3-68.7	34.5	0.9947	7.755	0.9838	0.587	13.45	5.70	6	6.97	Johnson (1986)
331	Butylbenzene	H	"	60.2	0.9999	13.14	0.9981	0.315	23.02	9.88	6	2.69	"
332	Ethylbenzene	H	"	47.2	0.9988	10.36	0.9947	0.407	18.10	7.74	6	9.29	"
333	Isopropylbenzene	H	"	52.3	0.9992	11.51	0.9957	0.355	20.11	8.60	6	5.03	"
334	Toluene	H	"	40.9	0.9973	9.151	0.9903	0.477	15.89	6.74	6	8.37	"
335	Anthracene	H	ACN/40-77.5	61.9	0.9937	16.50	0.9913	0.285	27.77	11.3	6	58.4	"
336	Phenanthrene	H	"	60.0	0.9949	15.93	0.9925	0.285	26.85	10.9	6	58.8	"
337	Pyrene	H	"	62.5	0.9962	16.68	0.9944	0.260	28.07	11.4	6	385	"
338	Benzene	H	MeOH/48.9-80	38.2	0.9948	4.719	0.9982	0.449	11.77	7.05	4	0.524	"
339	Butylbenzene	H	"	73.7	0.9900	9.098	0.9941	0.241	22.69	13.4	4	0.821	"
340	Isopropylbenzene	H	"	63.0	0.9904	7.819	0.9948	0.272	19.47	11.7	4	0.864	"
341	Toluene	H	"	47.4	0.9922	5.820	0.9967	0.365	14.56	8.74	4	0.620	"
342	Ethylbenzene	H	"	55.8	0.9911	6.895	0.9952	0.311	17.19	10.3	4	0.941	"
343	Biphenyl	C	MeOH/50-80	76.5	0.9993	6.900	0.9971	0.241	20.12	13.2	4	7.00	Woodburn (1985)
344	Phenanthrene	C	"	80.7	0.9996	7.490	0.9984	0.218	21.49	14.0	4	11.0	"
345	Anthracene	H	ACN/55-80	68.0	0.9949	12.76	0.9915	0.285	24.49	11.7	6	6.86	Lipford (1985)
346	Naphthalene	H	ACN/50-80	55.4	0.9894	10.76	0.9940	0.384	20.62	9.86	7	1.52	"

Column H: 15cmx4.6mm, 5μm Ultrasphere ODS(Altex); Column C: 5cmx4.6mm, 10μm Sepralyte C-8(Analytichem);
y-int.: y-intercept at ϕ_B=0; N: number of data point; α: F-test value

mechanism rather than partitioning, which is the basis of the assumption of equations 2-2 and 2-3. Another possibility is that the residual silanol groups are more accessible to solutes in short-chain stationary phases than for longer chains, and they may exert a strong effect on retention that is not treated in the partition model. Due to the significant curvature and the poor correlation found in this data set, we feel that the y-intercepts at ϕ_B=1 are unreliable, and therefore are not reported in Table 2-1. Where linearity is found to be good, it implies that the partition model, and the few assumptions applied are valid. This linearity is expected to be limited to the intermediate compositions explored here, because the random-mixing approximation assumed in the original derivation is expected to fail in the limits of extreme mobile phase composition of a few percent of either mobile phase component (Schoenmakers et al., 1983). In those cases, solutes or the minor component of the mobile phase may associate to form non-random mixtures.

According to equation 2-3, the slope of the regression of this composition plot equals the binary interaction constant characterizing the pair interaction between molecules of the A and B components in the mobile phase, multiplied by the size of the cavity occupied by the solute. Tests of this prediction of the cavity-size dependence are shown in Figures 2-3 and 2-4. The slopes of the composition plots are found to increase with increasing cavity size created by the solute molecules. That is, slope of $1/\phi_B \ln (k'/k_w)$ versus $\phi_B = n\chi_{AB}$ and

$$\partial \text{slope} \; / \; \partial n = \chi_{AB} \qquad (2\text{-}5)$$

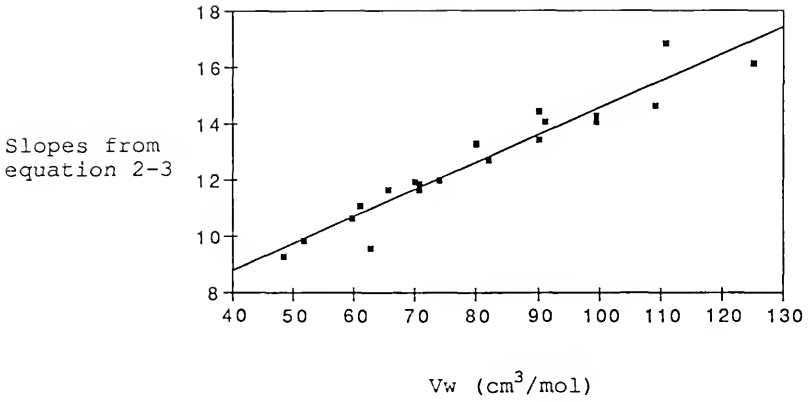


Figure 2-3. Plot of slopes from equation 2-3 versus van der Waals volume, Vw, of the solutes for the Sepralyte C-18 column with acetonitrile as modifier.

Table 2-2. Regression results of slopes from equation 2-3 versus the van der Waals volume, Vw, of the solutes for all the columns.

Column	Modifier	Slope($\times 10^2$)	r^2
Sepralyte C-2	Acetonitrile	16.51	0.9561
Sepralyte C-4	Acetonitrile	16.61	0.9263
Sepralyte C-8	Acetonitrile	7.817	0.7897
Sepralyte C-18	Acetonitrile	9.582	0.9164
Sepralyte C-4	Methanol	7.286	0.9189
Sepralyte C-8	Methanol	6.228	0.9305
Hypersil ODS	Acetonitrile	12.78	0.5499
Unisil Q C-18	Acetonitrile	8.640	0.4106
Ultrasphere ODS	Methanol	10.33	0.9977
Ultrasphere ODS	Ethanol	5.948	0.2490
Ultrasphere ODS	1-Propanol	4.662	0.0495
Ultrasphere ODS	Acetonitrile	18.57	0.8091
Silasorb C-8	Methanol	3.051	0.2339
Silasorb C-8	Acetonitrile	2.595	0.2083

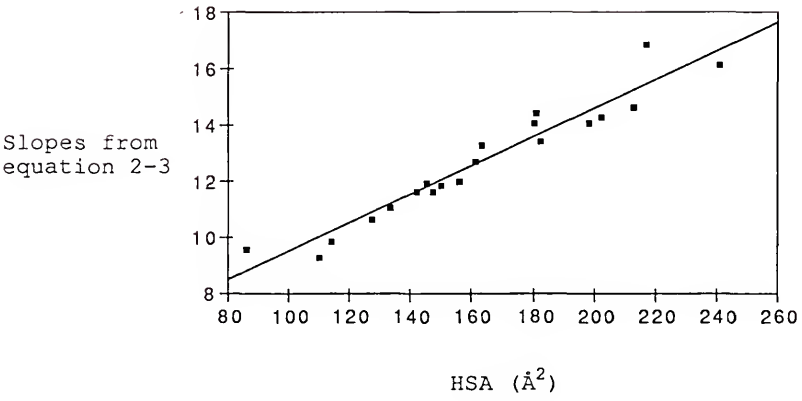


Figure 2-4. Plot of slopes from equation 2-3 versus hydrocarbonaceous surface area, HSA, of the solutes for the Sepralyte C-18 column with acetonitrile as modifier.

Table 2-3. Regression results of slopes from equation 2-3 vs the hydrocarbonaceous surface area, HSA, of the solutes for all the columns.

Column	Modifier	Slope($\times 10^2$)	r^2
Sepralyte C-2	Acetonitrile	8.511	0.9642
Sepralyte C-4	Acetonitrile	8.785	0.9465
Sepralyte C-8	Acetonitrile	4.115	0.8226
Sepralyte C-18	Acetonitrile	5.099	0.9280
Sepralyte C-4	Methanol	3.817	0.9068
Sepralyte C-8	Methanol	3.358	0.9520

where slope is defined as the slope of equation 2-3. Values of χ_{AB} generated from the derivative of equation 2-5 are presented in Tables 2-2 and 2-3. Due to the limited range of solute sizes available, this linearity is observed when either the cavity volume (Figure 2-3; slopes in Table 2-2), as measured by the van der Waals volume of the solute molecule or cavity area, as measured by the hydrocarbonaceous surface area of the solute molecule (Figure 2-4; slopes in Table 2-3), represented the size of the cavity created by the solute molecule. The binary interaction constants have different units depending on whether cavity size is taken to be that of the solute volume or area. The linear relationship holds well between the slopes from equation 2-3 and the cavity size; with most of these plots having r^2 of 0.9 and better. This linearity implies that the slopes from equation 2-3 are proportional to the size of the cavity occupied by the solutes. The slopes from Figures 2-2 and 2-3 represent the binary interaction constant of the mobile phase component A with B per unit volume or area respectively. For the Sepralyte C-18 column, shown in Figure 2-4 and Table 2-3, the interaction free energy of the mobile phase components is found to equal

$$(5.099 \times 10^{-2})kT = (5.099 \times 10^{-2})(592 \text{ cal/mol}) = 30.2 \text{ cal/mol } \text{Å}^2 \quad (2\text{-}6)$$

for these data taken at 25°C.

Several columns give poor correlations for the slope of equation 2-3 versus van der Waals volume of the solutes. For the case of the Hypersil column with acetonitrile as modifier, most of the solutes were nitrogenous compounds such as aniline, pyridine and amino-substituted

PAHs. It has been shown that many compounds with a nitrogenous moeity often exhibit an unsatisfactory degree of peak tailing in RPLC, and this effect is generally considered to come from the presence of residual silanol groups on the surface of the bonded phase (Wahlund and Sokolowski, 1978; Bayer and Paulus, 1987; Smith et al., 1986; Hansen et al., 1988). The retention mechanism of these compounds is most likely a mixed partition/adsorption process because of the interactions with the residual silanol groups, and would not be expected to fit the theory tested here. The Unisil Q C-18 data include many isomers of substituted benzene. The van der Waals volumes calculated from Bondi's (1964) method for geometric isomers are identical, but they give different slopes for equation 2-3. It has been shown in the literature that isomeric alkylbenzenes give relatively poor correlation with van der Waals volume (Smith 1981, Jinno and Kawasaki, 1983a). The poor correlation in this instance most likely arises from the misrepresentation of the calculated van der Waals volume, rather than a breakdown of the retention theory. The poor correlations for the ethanol and propanol data on the Ultrasphere ODS column may be caused by the changing stationary phase environment as these more hydrophobic modifiers are used in the mobile phase. There is not yet any theory published on the uptake of solvent by the stationary phase chains. Michels (1989) has recently reported that propanol appears to saturate the stationary phase at a very low mobile phase percentage of propanol. The poor correlations with the Silasorb column have yet to be accounted for.

The y-intercepts at ϕ_B=1 of equation 2-3 are predicted to equal the free energy of transfer of the solute between pure A and pure B of

the mobile phase, multiplied by the cavity size. That is, the y-intercepts of $1/\phi_B \ln (k'/k_w)$ versus ϕ_B at $(\phi_B=1) = n(\chi_{SB} - \chi_{SA})$ and

$$\partial \text{y-intercepts}(\phi_B=1) \; / \; \partial n = (\chi_{SB} - \chi_{SA}) \qquad (2\text{-}7)$$

The dependence of these intercepts on the cavity volume and area can be seen in Figures 2-5 and 2-6 respectively. The correlation coefficients of these intercepts and the cavity size are generally greater than 0.9, and the regression results are listed in Tables 2-4 and 2-5. The intercepts are found to increase linearly with cavity size as expected from the theory. Figure 2-6 shows a typical case, the Sepralyte C-18 column, with acetonitrile as component B and water as component A of the mobile phase. The exceptions noted are the same as those discussed above. The free energy of transfer of a solute per unit area can be calculated by utilizing Figure 2-6 and Table 2-5

$$(\chi_{SB} - \chi_{SA})kT = -(3.689 \times 10^{-2})(592 \text{ cal/mol}) = -21.8 \text{ cal/mol Å}^2 \qquad (2\text{-}8)$$

and is approximately constant for the same modifier used. The above tests confirm the predicted dependence of solute retention on cavity size, and the chromatographic method provides a convenient way to experimentally determine solute-solvent interaction free energies.

According to equation 2-3, the slope of the composition plot divided by n should give the binary interaction constant of the mobile phase components, χ_{AB}, regardless of the nature of the solutes and stationary phases used. This binary interaction constant should depend

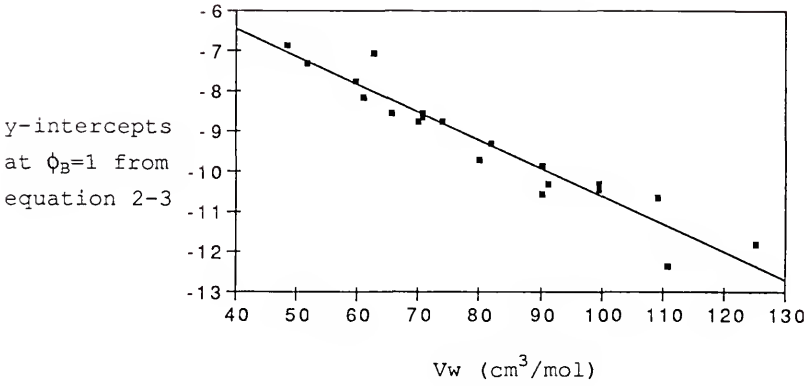


Figure 2-5. Plot of y-intercepts at ϕ_B=1 from equation 2-3 versus van der Waals volume, Vw, of the solutes for the Sepralyte C-18 column with acetonitrile as modifier.

Table 2-4. Regression results of y-intercepts at ϕ_B=1 from equation 2-3 versus the van der Waals volume, Vw, of the solutes for all the columns.

Column	Modifier	Slope(x10^2)	r^2
Sepralyte C-2	Acetonitrile	-8.523	0.9542
Sepralyte C-4	Acetonitrile	-7.484	0.9344
Sepralyte C-8	Acetonitrile	-9.793	0.9112
Sepralyte C-18	Acetonitrile	-6.936	0.9178
Sepralyte C-4	Methanol	-12.73	0.9489
Sepralyte C-8	Methanol	-12.17	0.9136
Hypersil ODS	Acetonitrile	-377.2	0.7089
Unisil Q C-18	Acetonitrile	-109.0	0.5035
Ultrasphere ODS	Methanol	-22.23	0.9993
Ultrasphere ODS	Ethanol	10.96	0.2696
Ultrasphere ODS	1-Propanol	95.35	0.1945
Ultrasphere ODS	Acetonitrile	-345.8	0.5705
Silasorb C-8	Methanol	721.2	0.2434
Silasorb C-8	Acetonitrile	629.9	0.2139

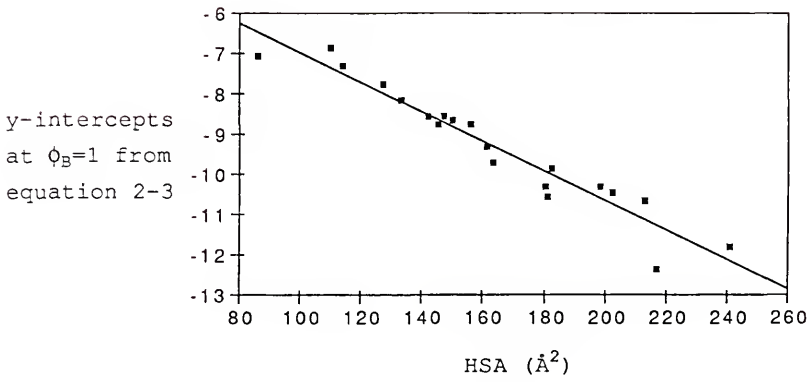


Figure 2-6. Plot of y-intercepts at ϕ_B=1 from equation 2-3 versus hydrocarbonaceous surface area, HSA, of the solutes for the Sepralyte C-18 column with acetonitrile as modifier.

Table 2-5. Regression results of y-intercepts at ϕ_B=1 from equation 2-3 vs the hydrocarbonaceous surface area, HSA, of the solutes for all the columns.

Column	Modifier	Slope($\times 10^2$)	r^2
Sepralyte C-2	Acetonitrile	-4.383	0.9658
Sepralyte C-4	Acetonitrile	-3.957	0.9389
Sepralyte C-8	Acetonitrile	-5.118	0.9052
Sepralyte C-18	Acetonitrile	-3.689	0.9282
Sepralyte C-4	Methanol	-6.752	0.9460
Sepralyte C-8	Methanol	-6.378	0.9489

solely on the nature of the organic modifier in the mobile phase of RPLC. By comparing the values of the slopes of Table 2-3, they are found to be independent of the solutes, but not the stationary phase. There is a factor of 2 differentiating the slopes from a short-chain stationary phase to a long-chain stationary phase, such as Sepralyte C-2 to the Sepralyte C-18 column. This variation can be attributed to two factors. First, in short-chain stationary phases, surface silanols are more accessible to solutes, and the interactions between them are much larger than in long-chain phases. Second, an alternate retention mechanism, adsorption (Dill 1987), should dominate in short-chain stationary phases. Both of these factors are neglected in the original partition model (Dill 1987).

Furthermore, the y-intercept at ϕ_B=1 divided by n should only be dependent on the nature of the solute and organic modifier. The data of the Sepralyte columns from Table 2-4 confirm this prediction since the same solutes are used for the Sepralyte columns. The large differences of the values in column 3 of Table 2-4 are mainly due to the differences in solutes employed with different stationary phases.

Johnson and coworkers (Johnson et al., 1986; Dorsey and Johnson, 1987) have shown in an earlier study that a solvatochromic dye molecule, referred to as ET(30), can be used to probe the chemical nature of the mobile phase and its strength as used in RPLC. The visible spectral shift of the dye is found to be linearly proportional to the binary interaction constant χ_{AB} of the mobile phase components (Figure 2-7). This provides a justification for the employment of ET(30) as a probe for the study of the mobile phase, since it appears to directly measure

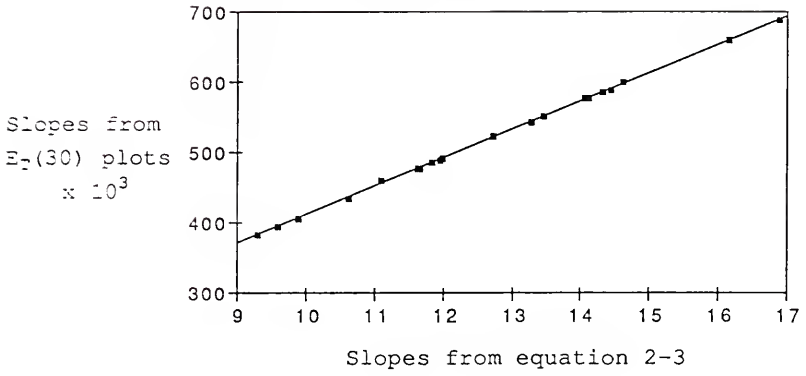


Figure 2-7. Plot slopes from $E_T(30)$ plots versus slopes from equation 2-3 for the Sepralyte C-18 column with acetonitrile as modifier.

Table 2-6. Regression results of slopes from equation 2-3 versus the slopes of $E_T(30)$ plots for all the columns.

Column	Modifier	Slope($\times 10^2$)	r^2
Sepralyte C-2	Acetonitrile	3.238	0.9996
Sepralyte C-4	Acetonitrile	2.699	0.9909
Sepralyte C-8	Acetonitrile	5.396	0.8796
Sepralyte C-18	Acetonitrile	4.047	0.9997
Sepralyte C-2	Methanol	14.67	0.9956
Sepralyte C-4	Methanol	9.315	0.9820
Sepralyte C-8	Methanol	11.03	0.9955
Silasorb C-8	Methanol	7.342	0.8369
Silasorb C-8	Acetonitrile	5.137	0.9987
Unisil Q C-18	Acetonitrile	4.913	0.9303
Hypersil ODS	Acetonitrile	3.671	0.9187
Ultrasphere ODS	Methanol	8.055	0.9997
Ultrasphere ODS	Ethanol	6.166	0.8827
Ultrasphere ODS	1-Propanol	2.467	0.2630
Ultrasphere ODS	Acetonitrile	4.066	0.8702

the free energy of contact between components A and B of the mobile phase.

For the Sepralyte C-18 column with acetonitrile as modifier, the slope of these two parameters are found to be

$$(4.047 \times 10^{-2}) \times (592 \text{ cal/mol}) = 23.96 \text{ units(cal/mol)} \qquad (2\text{-}9)$$

Other columns are observed to have the same linearity with other organic modifiers, and the linear regression results between these two parameters are listed in Table 2-6.

Even though the experimental data base used to test the theoretical predictions is relatively large, the range of mobile phase compositions is still limited to within 30 - 50% variation in solvent composition. The linear expression proposed by Dill (1987) and Dorsey and Dill (1989) tested in this work is found to hold well with the data base used. If the partition model and other assumptions are incorrect, nonlinearity should be observed. Since good linearity is found, the slopes and y-intercepts generated by plotting the composition plot of equation 2-3 should reflect, at least approximately, the binary interaction constants of the solutes and the solvents, and can be used to calculate the solute-solvent interaction free energies in RPLC.

CHAPTER III
CHARACTERIZATION OF THE RETENTIVITY OF REVERSED PHASE LIQUID CHROMATOGRAPHY COLUMNS

Introduction

There is an immense number of RPLC columns available on the market today. The majority of them are made from C_8 or C_{18} functional groups, but the use of columns having other functionalities, such as phenyl and cyano, has been on the rise. Recent studies done on commercial RPLC columns have found that they all showed significant differences in absolute retention and selectivity, α, for the same solute and mobile phase, even when they are all labelled as C_{18} columns (Goldberg 1982; Sander and Wise, 1988). This variability of RPLC columns is largely due to the difference in the starting silica as well as the bonding reaction (Dorsey and Dill, 1989). Since the stationary phase in RPLC has been shown to have ample effects on solute retention and selectivity (Sander 1988; Dill 1987; Sentell and Dorsey, 1989a; Sentell and Dorsey, 1989b), the variability has caused practical chromatographers many difficulties in choosing the best column to develop optimal separations. Developed methods are hard to transfer unless the brand and manufacturer of the column are specified.

Many studies have been performed by investigators to characterize the retention behavior of RPLC columns. Smith (1982a and 1982b) employed a homologous series of alkylarylketones to develop a retention

index with a set of reference compounds to define the retention performance of RPLC columns made from different manufacturers and functionalities. A different set of constants for each column is obtained for every different mobile phase composition used. These column retention constants are not very useful since new calibration on the column has to be done when mobile phase composition is changed. Antle and coworkers (Antle and Snyder, 1985; Antle et al., 1985) used gradient elution theory to characterize RPLC columns according to their solvophobic retention. They examined columns produced from the same base silica but having different bonded functionalities such as C_{18}, C_8, phenyl, C_1, or cyano groups. A large variety of test solutes having very diverse chemical structure were used in their study. They combined the volume phase ratio, Φ, and the polarity of a column into an effective phase ratio, J', which gives the retentivity of a column. A reference and a standard column were used to acquire the relative J' value of all columns studied. Their results revealed that the J' value of the columns are in the order of $C_{18} > C_8 >$ phenyl $> C_1 >$ cyano. They concluded that the contribution of the polarity of a column to the retentivity is small compared to the phase ratio of the column. Cooper and Lin (1986) have looked at the retention behavior of C_8, phenyl and cyano columns using three solutes chosen from Snyder's selectivity triangle (Snyder 1974; 1978). They found that the polarity of these columns is in the order of phenyl > cyano > C_8, and the overall retentivity of these columns is dominated by the phase ratio of the columns. Other researchers have used chemometric and factor analyses to characterize commercial columns (Delaney et al., 1987; Chretien et al.,

1986; Walczak et al., 1987), but only qualitative results were obtained in these studies. Walczak et al. (1987) concluded that carbon loading, nature of the organic ligands, and the accessibility of the surface silanol groups are the main factors governing the retentivity and selectivity of the columns. Delaney et al. (1987) summarized that their classification of RPLC columns from chemometric results agreed well with a qualitative scheme developed by a liquid chromatography specialist.

The present study provides a simple method to characterize the retentivity of commercial RPLC columns. Binary mobile phases of water and an organic modifier under isocratic conditions were used throughout the study to avoid lengthy equation derivation, and to keep experimental parameters simple. No reference or standard column was needed, and solutes of many different types were employed so that the results of the study should be applicable to all solutes.

Experimental Section

All the retention measurements were obtained either with a Spectra-Physics SP8700 ternary proportioning LC system (Spectra-Physics, San Jose CA, USA) or with two Spectroflow 400 pumps (ABI Analytical, Kratos Division, Ramsey, NJ, USA). A Valco liquid chromatography injection valve (Valco Instrument Company, Houston, TX, USA) and a 20 µl sample loop were used to inject the solutes. The detection system was either a Spectroflow 783 absorbance detector/gradient controller (ABI Analytical, Kratos Division, Ramsey, NJ, USA) operated at 254 nm or a Spectroflow 757 absorbance detector (Kratos Analytical Instruments, Ramsey, NJ, USA) also operated at 254 nm. When the two Spectroflow 400 pumps were used, a Rainin dynamax dual chamber mixer (Rainin Instrument

Company, Inc., Woburn, MA, USA) was placed before the injection valve for better mixing of the binary solvents. All the Zorbax columns were commercially available from Du Pont (E. I. du Pont de Nemours and Company, Wilmington, DE, USA), the B & J columns from Burdick & Jackson Laboratories, Inc. (Baxter Healthcare Corp., Burdick & Jackson Division, Muskegon, MI, USA), and the Ultrasphere column from Beckman (Beckman Instruments, Inc., Fullerton, CA, USA). The high density column was synthesized and packed in our laboratories (Sentell 1987). Some of the properties of these columns as supplied by the manufacturer are listed in Table 3-1. A Fisher Recordall Series 5000 (Fisher Scientific, Pittsburgh, PA, USA) strip chart recorder was used to record all the chromatographic peaks. The solvents used were HPLC grade from Fisher Scientific (Fisher Scientific, Pittsburgh, PA, USA), and the water was filtered through a Barnstead NANOpure II system (Barnstead Company, Dubuque, IA, USA) before being used. The columns were maintained at 30°C by a water jacket and a Haake D1 circulator (Haake, Dieselstrasse, West Germany).

A total of 26 solutes was used and they are listed in Table 3-2. They were from various suppliers and were used without further purification. The solutes were grouped into mixtures containing no more than 5 solutes based on their retention data when they were injected individually at the beginning of the study. Peak assignments were accomplished by identifying the peak area and elution order. The void volume of each column was determined by the injection of water at mobile phase composition of 75% water and 25% organic modifier. The flow rate was maintained at 1 ml/min except for the Burdick & Jackson C-18 column which was used with a flow rate of 1.5 ml/min. All the retention data

Table 3-1. Properties of the RPLC columns as supplied by the manufacturers.

Column	Ligand	Surface area(m^2/g)	% Carbon loading
Zorbax ODS	C_{18}	340	20
Zorbax C-8	C_8	330	10
Zorbax phenyl	Phenylpropyl	300	5
Zorbax TMS	C_1	320	5
Zorbax cyano	Cyanopropyl	320	5
B & J C-8	C_8	250	8
B & J C-18	C_{18}	250	13
Ultrasphere C-8	C_8	200	12

Table 3-2. List of test solutes used in this study.

Methyl paraben	Ethyl paraben
Propyl paraben	Butyl paraben
Cortisone	Corticosterone
Toluene	Hexylfluorobenzene
Benzyl Alcohol	o-Nitrophenol
Propachlor	1-Methyl naphthalene
Tri-p-tolyl phosphate	Methyl benzyl amine
Fluorobenzene	Chloropropham
Dimethyl phthalate	Diethyl phthalate
Butyl benzyl phthalate	Dibutyl phthalate
1-Methyl phenanthrene	Dioctyl phthalate
p-Terphenyl	Chrysene
1,1,4,4,-Teraphenyl-1,3-butadiene	Octadecanophenone

were obtained from averaging at least two measurements of a solute. The $E_T(30)$ polarity values for the different mobile phase compositions were calculated from the quadratic relationship between percent organic modifier and the $E_T(30)$ values reported by Dorsey and Johnson (1987).

The regression calculations were all done by using the StatWorks (Heydon and Son Inc., Philadelphia, PA, USA) program on a Macintosh SE (Apple Computer, Inc., Cupertino, CA, USA) microcomputer.

Results and Discussion

Since the composition of the mobile phase plays a primary role in the retention of solutes in RPLC, it is important to have a method to account for the solute retention contributed by the mobile phase before the retentivity of the stationary phase can be explored. Many workers have used solvatochromic measurements to measure the polarity of the mobile phase used in RPLC, such as the "Z" scale (Kosower 1958), the π^* multiparameter scale (Taft and Kamlet, 1976; Kamlet et al., 1983; Taft et al., 1985; Sadek et al., 1985), and the $E_T(30)$ scale (Johnson et al., 1986; Dorsey and Johnson, 1987). Johnson and coworkers (Johnson et al., 1986; Dorsey and Johnson, 1987) have shown that the ln k' of a solute in RPLC is linearly associated with the $E_T(30)$ polarity scale and has better linearity than the volume percent of organic modifier. Their relationship can be expressed as

$$\ln k' = m (E_T(30)) + c \qquad (3\text{-}1)$$

where $E_T(30)$ is the polarity value in kcal/mol of the mobile phase measured by the $E_T(30)$ scale, and m and c are the slope and y-intercept of the linear regression. The $E_T(30)$ values are found to have an excellent quadratic relationship with most common binary mobile phases used in RPLC (Dorsey and Johnson 1987). Moreover, the slope m has been shown in Chapter II to be directly proportional to the size of the solutes and the binary interaction constant of the mobile phase. We selected to employ the slope m from equation 3-1 as the descriptor for the mobile phase contribution to solute retention.

Molecular Descriptor Approach

Although the mobile phase effect can be accounted for by using the slope in equation 3-1, the cavity created in the stationary phase also is a major contribution to solute retention according to the partition model. The first approach we took in this study was to search for a molecular descriptor of the test solutes that can delineate the cavity in the stationary phase, and use it to plot against slope m. The resulting slope of this plot should only be a function of the retentivity of the column. We first predicted that a linear relationship should occur between the slopes from equation 3-1 and a molecular size descriptor. It has been shown in the literature that a linear dependence exists between ln k' of a solute and some molecular size descriptors of the solute such as the van der Waals volume, Vw, (Hanai and Hubert, 1984a; Smith 1981; Jinno and Kawasaki, 1983a) and the molecular connectivity index, χ_1 (Wells et al., 1982; 1986). Slopes from equation 3-1 were plotted against both the van der Waals volume and the molecular connectivity index (Figures 3-1 and 3-2) and the

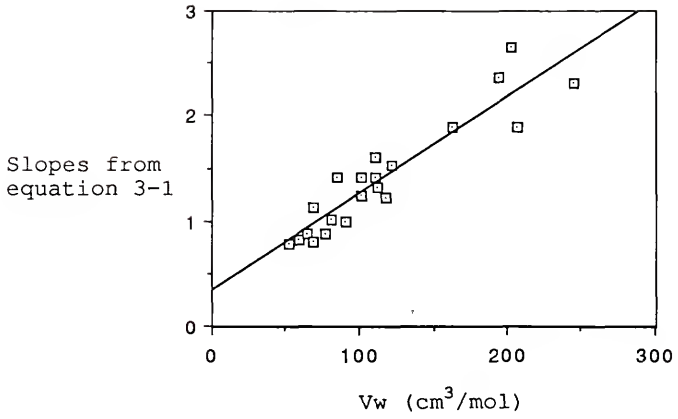


Figure 3-1. Plot of slopes from equation 3-1 against the van der Waals volume, Vw, of the test solutes for the Zorbax TMS column using acetonitrile as modifier.

Table 3-3. Regression results of graphs of slopes from equation 3-1 against van der Waals volume, Vw, of the test solutes.

Column	Modifier	Slopex(10^3)	y-inter.	r^2
Zorbax ODS	ACN	4.08	0.845	0.533
Zorbax C-8	ACN	3.82	1.03	0.534
Zorbax phenyl	ACN	4.27	0.593	0.484
Zorbax TMS	ACN	9.25	0.336	0.861
Zorbax cyano	ACN	6.98	0.310	0.472

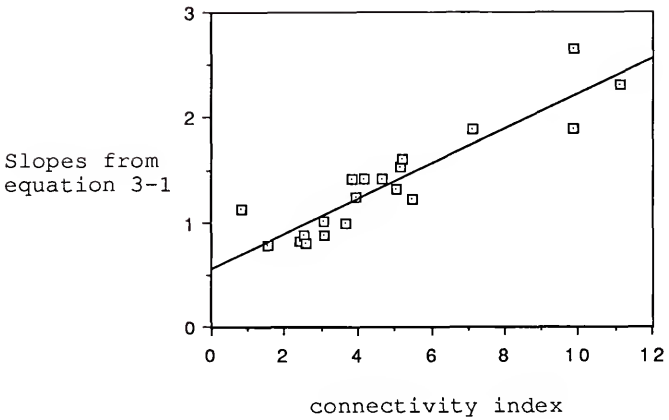


Figure 3-2. Plot of slopes from equation 3-1 against the molecular connectivity index of the test solutes for the Zorbax TMS column using acetonitrile as modifier.

Table 3-4. Regression results of graphs of slopes from equation 3-1 against molecular connectivity index of the test solutes.

Column	Modifier	Slopex(10^2)	y-inter.	r^2
Zorbax ODS	ACN	6.21	0.992	0.401
Zorbax C-8	ACN	6.54	1.14	0.417
Zorbax phenyl	ACN	7.14	0.723	0.379
Zorbax TMS	ACN	16.7	0.563	0.829
Zorbax cyano	ACN	11.5	0.531	0.354

regression results of these graphs on five Zorbax columns are shown in Table 3-3 and 3-4 respectively.

The coefficients of determination, r^2, of these regressions are all well below 0.9 meaning that there is no significant correlation between the slopes from equation 3-1 and the two molecular size descriptors of the solutes that we have chosen. One of the reasons for the breakdown of our initial hypothesis is probably because the molecular size descriptors do not account for all the interactions among the solutes and the stationary phase. Despite Chapter II showing that the cavity opened in the stationary phase is proportional to the size of the solute, there are other chemical interactions such as polarity, dipole moment, and hydrogen bonding ability between the solute and the stationary phase. These interactions cannot be completely summed by one single molecular size descriptor.

ln k_w Approach

The next approach we took was to seek a parameter that will accurately include all the interactions between the solute and the stationary phase. The logarithmic capacity factor of a solute at 100% water, ln k_w, has been shown to have great correlation to the logarithm of the water/octanol partition coefficient, log $P_{o/w}$, of a solute (Miyake et al., 1988; Braumann 1986; Braumann et al., 1987; Minick et al., 1987). The linear relationship shows that both ln k_w and log $P_{o/w}$ are measuring a similar partition process. This suggests that ln k_w measures the driving force of the partition of a solute into the stationary phase, and is the parameter that measures all the interactions between the solute and the stationary phase.

The ln k_w of a solute is difficult to obtain experimentally in RPLC due to long retention times and poor peak shapes arising from slow mass transfer with pure water as the mobile phase. There is a popular belief that the logarithm of the capacity factor, ln k', of a solute is linearly related to the percent by volume of organic modifier in the mobile phase (Snyder et al., 1979; Dolan et al., 1979; Schoemakers et al., 1979; Antle et al., 1985; Baty and Sharp, 1988). Hence, ln k_w is often estimated by extrapolation from a linear regression of ln k' versus percent organic modifier back to 0% organic modifier (Reymond et al., 1987; Braumann 1986; Braumann et al., 1987; Baty and Sharp, 1988).

$$\ln k_w = S\ (\%\text{organic}) + c' \qquad (3\text{-}2)$$

Unfortunately, this kind of extrapolation has been found to be not as reliable as using the $E_T(30)$ polarity scale (Michels 1989). The $E_T(30)$ polarity value of pure water is 63.11 kcal/mol, and therefore ln k_w of a solute can be approximated by substituting 63.11 kcal/mol into equation 3-1.

Since the slopes from equation 3-1 and the ln k_w of the solutes summarize the retention effects due to the mobile phase and the solute interactions with the stationary phase respectively, the slope of a plot of these two parameters for a column should be independent of the two effects, and unveil the retentivity of the column (Figure 3-3). The regression results of these plots for the columns studied are presented in Table 3-5. The 95% confidence interval of the slopes of these

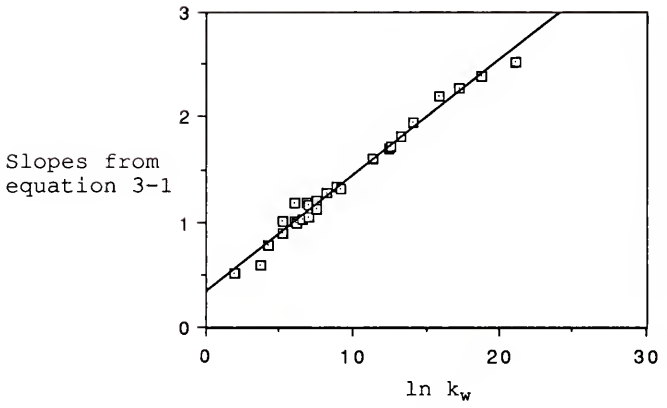


Figure 3-3. Plot of slopes from equation 3-1 against the ln k_w of the test solutes for the Zorbax TMS column using acetonitrile as modifier.

Table 3-5. Regression results of slopes from equation 3-1 vs ln k_w of the test solutes for all the columns.

Column	Modifier	Slope	y-inter.	r^2	CI@$t_{0.05}$[a]
Zorbax ODS	ACN	0.0835	0.525	0.894	±0.004
Zorbax C-8	ACN	0.069	0.727	0.834	±0.035
Zorbax Phenyl	ACN	0.104	0.293	0.98	±0.005
Zorbax TMS	ACN	0.110	0.349	0.982	±0.007
Zorbax Cyano	ACN	0.126	0.241	0.99	±0.005
Zorbax ODS	MeOH	0.120	0.320	0.977	±0.003
Zorbax C-8	MeOH	0.124	0.310	0.987	±0.004
Zorbax Phenyl	MeOH	0.129	0.271	0.993	±0.003
Zorbax TMS	MeOH	0.134	0.346	0.979	±0.006
Zorbax Cyano	MeOH	0.130	0.362	0.96	±0.006
B & J C-8	MeOH	0.134	0.209	0.987	±0.009
B & J C-18	MeOH	0.114	0.326	0.945	±0.012
Ultrasphere C-8	MeOH	0.122	0.307	0.99	±0.004
High Density	MeOH	0.115	0.314	0.923	±0.013

a) 95% confidence interval of the slopes of the regressions

regressions are calculated (Sharaf et al., 1986; Anderson 1987), and they are listed in the last column of Table 3-5. The confidence interval shows that almost all the slopes of the regressions are statistically different from one another. Good linearity appears between these two parameters with the r^2 of all the columns tested above 0.9, except in the case of the Zorbax ODS and C-8 columns with acetonitrile as modifier. The high r^2 confirms that the slopes from equation 3-1 and ln k_w of the test solutes are highly correlated. This type of slope versus ln k_w plot is not new. Several groups (Braumann et al., 1983; Hammer et al., 1982) have shown that plots of the slope, S, and ln k_w from equation 3-2 are linear. They found the slopes and y-intercepts of these plots formed two empirical parameters that can be employed to classify solutes into different groups. These solute groups can be used to recommend the use of ln k_w for the estimation of log $P_{o/w}$. Schoenmakers et al. (1979) found good linearity when methanol is used as mobile phase modifier but not with acetonitrile or THF. They have shown that when linearity is observed, the slopes of these plots can be used to determine the shape of the gradient program. Moreover, these slopes can be employed to predict isocratic capacity factors from a simple gradient analysis (Schoenmakers et al., 1981). Baty and Sharp (1988) observed good correlation between S and ln k_w for methanol, acetonitrile and THF using structurally similar solutes. They tried to use the slopes and y-intercepts of these plots to predict capacity factors of the solutes, but they found that the slopes and y-intercepts of these plots vary with the nature of the organic modifiers and

columns. Therefore, the chromatographic system and the solute group have to be defined before the capacity factors can be predicted.

The general retentivity of the columns in this study for a given organic modifier is found to be inversely proportional to the slope of the slopes versus ln k_w plot. The usefulness of this relative retentivity scale can be seen by plotting the linear regressions of the five Zorbax columns using acetonitrile as modifier on one graph (Figure 3-4). As shown in Chapter II, the slopes from equation 3-1 are found to be proportional to the size of the cavity created by the solutes; therefore, at a given solute cavity size, the linear regression having the smallest slope will have the largest ln k_w and hence, the largest retentivity. From the data shown in Figure 3-4, the Zorbax cyano column has the largest slope followed by the TMS column, phenyl column, ODS column and C-8 column; retentivity of these column is in the order of C-8 > ODS > phenyl > TMS > cyano. The Zorbax C-8 column seems to have a higher retentivity than the Zorbax ODS column when acetonitrile is used as modifier. A careful examination of the confidence interval of these two slopes demonstrates that they actually have no statistical difference, and the apparent higher retentivity of the Zorbax C-8 column could very well be an experimental artifact. The apparent stronger retentivity of the Zorbax cyano column over the TMS column with methanol as modifier can be attributed to the hydrogen bonding between the surface silanols and the methanol. Cyano columns have been shown to have more accessible silanols than other columns (Cooper and Lin, 1986; Smith and Miller, 1989). Since methanol can form hydrogen bonds with these silanols, the surface of cyano columns is significantly modified

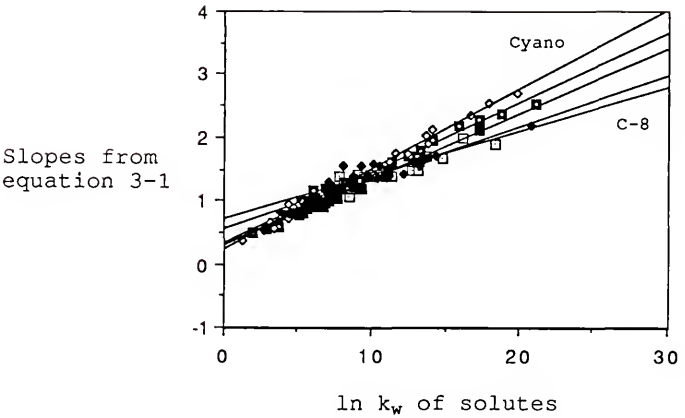


Figure 3-4. Plot of slopes from equation 3-1 against the ln k_w of the test solutes for all the Zorbax columns using acetonitrile as modifier.

by the methanol, and hence its apparent retentivity is stronger than the TMS column. When acetonitrile is used as modifier, due to its lack of hydrogen bonding ability, the retentivity of the TMS column is found to be stronger than the cyano column. Other commercial columns can be tested using the same procedures and compared with the retentivity of the Zorbax columns. With methanol as modifier, the B & J C-18 column is found to have retentivity larger than any of the Zorbax columns, and the B & J C-8 column is found to have retentivity approximately the same as the Zorbax TMS column, while the Ultrasphere C-8 column is shown to have retentivity between the Zorbax ODS and C-8 columns.

One interesting point raised by many researchers is the importance of phase ratio of the column on the total retentivity of the column. The significance of phase ratio on retentivity was also investigated in this study. Phase ratio in RPLC can be calculated using the volume ratio of the stationary phase to the mobile phase. Although much work has been done on measuring the volume of the mobile phase, V_m, (Melander et al., 1983; Smith et al., 1986; Engelhardt et al., 1984; Hennion and Rosset, 1988) measurement of the volume of the stationary phase, V_s, is more ambiguous (Jandera et al., 1982; Melander et al., 1980; Sander and Field, 1980; Slaats et al., 1981; McCormick and Karger, 1980a; Berendsen et al., 1980). By employing the V_s and V_m calculation method presented by Sentell (1987), the phase ratios of the columns studied were obtained and are listed in Table 3-6. In the case of the phenyl and cyano columns, the density of the ligands are not available and the V_s of the columns cannot be calculated. The phase ratio of these columns are therefore not reported in Table 3-6. With the exception of

Table 3-6. Phase ratio of the columns in this study calculated using the method presented by Sentell (1988).

Column	Phase ratio
Zorbax ODS	0.367
Zorbax C-8	0.226
Zorbax phenyl	unknown
Zorbax TMS	0.172
Zorbax cyano	unknown
B & J C-8	0.0619
B & J C-18	0.110
Ultrasphere C-8	0.380
High density	0.465

the two B & J columns, the phase ratio of the columns displays the same order as the relative retentivity scale of the columns. The high density column, which has the largest phase ratio, is found to be close to having the highest retentivity. This result points out that the phase ratio of a column indeed has a vital contribution to the overall retentivity of the column.

This work exhibits a simple method that can be used to classify commercial RPLC columns according to their overall retentivity. The isocratic approach used has kept the data interpretation and manipulation simple. The use of ln k_w in the procedure is justified and is found to be very appropriate. The phase ratio of a column is shown to dominate the total retentivity of the column. Since such a large variety of test solutes were employed in this work, the relative retentivity scale based on this study should be applicable to almost all solutes.

CHAPTER IV
CONCLUSIONS AND FUTURE WORK

Conclusions

The research that has been discussed in the previous chapters was performed to confirm and apply the partition retention mechanism of RPLC. The recognition of the partition mechanism as the correct retention model in RPLC is very important because it can help chromatographers to understand the processes happening in RPLC. Also, with a sound fundamental understanding of solute retention, more robust separation methods can be developed.

In Chapter II, we have tested the partition model with a large data base. We found that the relationships between solute capacity factors and mobile phase compositions are in good agreement with the prediction from the partition model (Dill 1987; Dorsey and Dill, 1989) with only very minor discrepancies. The partition model proposed by Dill (1987) is based on simple lattice models and the interphase model of the stationary phase, but the results of our tests are still very good. With a more explicit treatment of the simple assumptions, the theory may be able to account for the small discrepencies. The molecular origins of these composition plots are also clarified in our tests. The slopes of these plots are found to be a function of the binary interaction parameter of the mobile phase components, and the y-intercepts at ϕ_B=1 are proportional to the free energy of transfer of the solutes. The solute size plots provide a method to calculate the

solute-solvent interaction free energies, and an explanation to the widely observed relationship between capacity factors and solute sizes in RPLC (Hanai and Hubert, 1984b; Jinno and Kawasaki, 1983a; 1983b; Feng et al., 1988; Mockel et al., 1987). Moreover, the slopes and y-intercepts at ϕ_B=1 of the composition plots predicted by the theory give a simple and reliable method to estimate solute-solvent interaction free energies. These free energies are difficult to obtain experimentally and are seldom reported in the literature. With this new approach, these interaction free energies should be much easier to access due to the availability of chromatographic data.

The partition model is put to practice in Chapter III. We employed the partiton model as our basis to develop a relative retentivity scale for RPLC columns. In the partiton model, the entire stationary phase contribution to retention is summed in the ln k_w term of the solutes. Although this assumption is an oversimplification of the stationary phase effect, it has been shown that ln k_w is a very effective parameter to estimate the solute partition coefficient (Miyake et al., 1988; Braumann 1986; Braumann et al., 1987; Minick et al., 1989). We used the ln k_w of the solutes and the slopes from the ln k' versus $E_T(30)$ values plots to obtain the relative retentivity values of the RPLC columns. The correlation between these two parameters is shown to be good, and this further supports the partition model as the dominant solute retention mechanism. The retentivity scale is found to be in good agreement with the literature (Antle and Snyder, 1985; Antle et al., 1985). Also, the experimental procedures are kept relatively simple, and therefore this retentivity scale should be useful in

classifying the retentivity of commercial RPLC columns, and should help practical chromatographers to select the best column for their applications. Since test solutes of various chemical structures are employed in our study, the retentivity scale should be applicable to all solutes. The phase ratio of the columns is found to play a major role in determining the retentivity of the column.

Future Work

Although the data base that is used in Chapter II to test the predictions from the partition model is rather large, it lacks a wide range in mobile phase compositions especially at the extreme compositions. Most of the data sets have mobile phase composition range between 30 - 50% organic modifier. A test of the predictions at extreme mobile phase compositions such as mobile phases having a few percent of organic modifier would be interesting, since it will unveil how well the theory holds under extreme mobile phase composition. The stationary phase has been reported to take on a different structure as the composition of the mobile phase changes (Yonker et al., 1982a; 1982b; McCormick and Karger, 1980a; 1980b; Carr and Harris, 1986; 1987; McNally and Rogers, 1985). The changing in the structure of the stationary phase implies that the retention mechanism may switch from partition to other forces. By testing the theory at extreme compositions, the mobile phase composition that can significantly change the stationary phase structure may be observed as the relationship becomes nonlinear.

Another test that is useful in testing the validity of the partition model is to use retention data from stationary phases having functionalities other than alkyl chains, such as phenyl and cyano

groups. The data from these stationary phases should give information on the retention mechanism in these different phases. If linearity exists in these columns, partition should be the dominate force. If a nonlinear relationship occurs, a retention mechanism other than partition should be considered for these columns.

The experimental procedures for obtaining the relative retentiviity scale in Chapter III is simple; nevertheless it is rather time consuming since isocratic data of more than 20 solutes have to be collected. A possible time saving step is to find a few representative solutes so as to eliminate the useage of all 26 test solues. The Snyder selectivity triangle (Snyder 1974; 1978) provides a good starting place to look for representative solutes.

Moreover, the present study only involved monomeric coverage columns. In order to expand the practical utility of the retentivity scale, stationary phases other than monomeric coverage should also be tested to find out if they fit in the retentivity scales. Since polymeric phases have been shown to have better selectivity for PAH's than monomeric phases (Sander and Wise, 1984), and more and more polymeric phases are available, there is a need to classify and compare them with the monomeric phases. By using polymeric columns, the applicability of our retentivity scale can be tested. Also, since the retentivity scale is based on the partition model, a failure in classifying polymeric columns with the retentivity scale may suggest that a retention mechanism other than partition is the dominate factor in polymeric phases. The surface coverage of polymeric phases is usually higher than monomeric phases. It has been pointed out that these reported values may not give the true surface coverage for

polymeric phases (Sander and Wise, 1984; Dorsey and Dill, 1989), because during the polymerization process, it is very likely that a reactive silane molecule will bond at a point away from the silica surface. The validity of these reported values may be revealed by combining the phase ratio and the retentivity values of these polymeric columns.

APPENDIX A
CHROMATOGRAPHIC RETENTION DATA

Column: Zorbax ODS
Mobile phase: ACN/water
Reference: This work

Solute	ln k' at volume % of acetonitrile in the mobile phase										
	85	80	75	70	65	60	50	40	35	30	25
Methyl Paraben							-0.93	-0.05	0.40	0.87	1.37
Ethyl Paraben						-1.13	-0.20	0.67	1.13	1.66	
Propyl Paraben						-0.39	0.45	1.39	1.91		
Butyl Paraben				-0.57		0.20	1.05	2.08	2.66		
Cortisone							-1.21	-0.04	0.57	1.30	2.16
Corticosterone							0.16	1.08	1.63	2.32	
Toluene						0.90	1.60	2.40	2.83	3.42	
Hexylfluorobenzene		-0.69		0.13		0.90	1.70	2.61			
Benzyl Alcohol								-0.48	-0.10	0.25	0.65
o-Nitrophenol						-0.10	0.52	1.29		2.08	
Propachlor						0.14	0.89	1.76		2.80	
1-Methyl naphthalene		0.26		0.94		1.66	2.50	3.51			
Tri-p-toyly phosphate	0.18			0.61		2.60	3.83				
Methyl benzyl amine				-1.23		-0.50	0.16	0.83	1.20		
Fluorobenzeze				-0.35		0.34	1.04	1.78			
Chloropropham		-0.85		-0.01		0.80	1.66	2.68			
Dimethyl phthalate				-1.61		-0.62	0.16	0.92			
Diethyl phthalate			-0.88	-0.49		0.32	1.13				
Dibutyl phthalate	-0.50	0.41		1.23		2.13	3.17				
1-Methyl phenanthene	0.52	0.91	1.29	1.64		2.44	3.50				
Butyl benzyl phthalate	-0.33	0.18		1.05		1.99	3.09				
Dioctyl phthalate	2.58	3.17	3.75	4.35							
p-Terphenyl		1.19		2.04	2.49	2.99					
Chrysene		1.34		2.10	2.52	2.96					

Column: Zorbax C-8
Mobile phase: ACN/water
Reference: This work

Solute	ln k' at volume % of acetonitrile in the mobile phase									
	80	75	70	65	60	50	40	30	25	20
Methyl Paraben					-2.24	-1.01	-0.04	0.91	1.44	
Ethyl Paraben					-1.19	-0.29	0.66	1.71	2.30	
Propyl Paraben			-1.46		-0.49	0.35	1.35			
Butyl Paraben			-0.77		0.06	0.92	2.01			
Cortisone						-1.79	-0.35	1.08	1.96	2.94
Corticosterone					-1.53	-0.50	0.55	1.94	2.81	
Toluene			0.00		0.68	1.47	2.24			
Hexylfluorobenzene			0.10	0.49	0.86	1.69	2.63			
Benzyl Alcohol						-1.46	-0.61	0.19	0.61	1.03
o-Nitrophenol			-1.19		-0.47	0.36	1.10			
Propachlor			-0.77	-0.44	-0.06	0.79	1.70			
1-Methyl naphthalene		0.22	0.59	0.92	1.30	2.20	3.25			
Tri-p-toyly phosphate	0.51	0.98	1.45	1.91	2.44					
Methyl benzyl amine			-1.48		-0.79	0.01	0.74			
Fluorobenzeze			-0.48		0.17	0.93	1.73			
Chloropropham			-0.03	0.33	0.74	1.66	2.75			
Dimethyl phthalate			-1.62	-1.25	-0.76	0.11	0.96			
Diethyl phthalate			-0.48	-0.14	0.25	1.11	2.07			
Dibutyl phthalate	0.32	0.77	1.18	1.59	2.06	3.16				
1-Methyl phenanthrene		0.74	1.11	1.48	1.91	2.91				
Butyl benzyl phthalate	0.08	0.56	1.00	1.44	1.91	3.07				
Dioctyl phthalate	2.85	3.45	4.02	4.63						
p-Terphenyl	0.55	1.01	1.47	1.88						
Chrysene	0.53	0.96	1.36	1.79						

Column: Zorbax cyano
Mobile phase: ACN/water
Reference: This work

Solute	ln k' at volume % of acetonitrile in the mobile phase									
	70	60	55	50	40	35	30	25	20	15
Methyl Paraben					-0.74	-0.30	0.09	0.45	0.88	
Ethyl Paraben					-0.26	0.17	0.59	1.00	1.46	
Propyl Paraben				-0.72	0.19	0.67	1.12	1.59		
Butyl Paraben				-0.36	0.61	1.12	1.64	2.16		
Cortisone						-0.41	0.17	0.77	1.41	
Corticosterone						0.05	0.62	1.22	1.91	
Toluene				-0.17	0.59	0.98	1.30			
Hexylfluorobenzene		-0.81		0.17	0.92	1.35	1.67			
Benzyl Alcohol							-0.67	-0.38	-0.10	0.17
o-Nitrophenol					0.08	0.41	0.73	0.97		
Propachlor				-0.52	0.26	0.71	1.12	1.54		
1-Methyl naphthalene		-0.51		0.38	1.30	1.85	2.34	2.84		
Tri-p-toyly phosphate	0.18	-0.11		1.08	2.43	3.39				
Methyl benzyl amine					-0.37	-0.03	0.30	0.57	0.87	
Fluorobenzeze				-0.33	0.35	0.71	1.02	1.26		
Chloropropham				0.12	1.05	1.62	2.12	2.60		
Dimethyl phthalate				-1.04	-0.20	0.19	0.56			
Diethyl phthalate				-0.41	0.44	0.91	1.35			
Dibutyl phthalate	-1.80	-0.41		0.65	1.88					
1-Methyl phenanthrene	-1.14	-0.08		0.88	1.94	2.66				
Butyl benzyl phthalate		-0.09	0.46	1.02	2.20					
Dioctyl phthalate	-0.56	0.76		2.19	3.97					
p-Terphenyl	-0.78	0.35	0.88	1.47	2.74					
Chrysene	-0.61	0.40	0.89	1.43	2.62					
1,1,4,4,-Teraphenyl-1,3-butadiene	-0.28	0.98	1.62	2.34						
Octadecanophenone	0.19	1.58	2.31	3.19						

Column: Zorbax phenyl
Mobile phase: ACN/water
Reference: This work

Solute	ln k' at volume % of acetonitrile in the mobile phase								
	80	75	70	65	60	50	40	30	20
Methyl Paraben					-1.01	-0.43	0.14	0.81	
Ethyl Paraben					-0.74	-0.10	0.56	1.33	
Propyl Paraben			-0.95		-0.40	0.25	1.02	1.94	
Butyl Paraben			-0.74		-0.10	0.42	1.47	2.53	
Cortisone					-1.14	-0.63	0.17	1.05	
Corticosterone			-0.94		-0.47	0.05	0.83	1.73	
Toluene			-0.17		0.31	0.91	1.65	2.29	
Hexylfluorobenzene			-0.10		0.51	1.19	2.04	2.70	
Benzyl Alcohol						-0.72	-2.82	0.21	0.99
o-Nitrophenol			-0.76		-0.25	0.21	0.82	1.44	
Propachlor			-0.43		0.05	0.63	1.36	2.24	
1-Methyl naphthalene			0.22		0.84	1.51	2.43	3.47	
Tri-p-toyly phosphate	-0.05		0.75		1.67	2.67			
Methyl benzyl amine			-0.94		-0.36	0.08	0.66	1.26	
Fluorobenzeze			-0.51		0.07	0.60	1.29	1.95	
Chloropropham			-0.29		0.46	1.15	2.09	3.11	
Dimethyl phthalate			-0.90		-0.32	0.22	0.90	1.45	
Diethyl phthalate			-0.46		0.18	0.82	1.67	2.26	
Dibutyl phthalate	-0.30		0.46		1.26	2.17			
1-Methyl phenanthrene	-0.06		0.57		1.30	2.10	3.27		
Butyl benzyl phthalate			0.14	0.67	1.17	2.30			
Dioctyl phthalate	0.99		2.03		3.20	4.61			
p-Terphenyl	-0.46	0.10	0.58	1.10	1.60	2.76			
Chrysene	-0.50	0.01	0.46	0.94	1.41	2.47			
1,1,4,4,-Teraphenyl-1,3-butadiene	0.68	1.26	1.80	2.40					
Octadecanophenone	1.22	1.88	2.52	3.22					

Column: Zorbax TMS
Mobile phase: ACN/water
Reference: This work

Solute	ln k' at volume % of acetonitrile in the mobile phase									
	80	70	65	60	50	40	35	30	25	20
Methyl Paraben					-1.51	-0.50	-0.13	0.31	0.75	
Ethyl Paraben					-0.86	0.04	0.44	0.92	1.40	
Propyl Paraben					-0.36	0.57	1.02	1.54		
Butyl Paraben					0.07	1.06	1.55	2.14		
Cortisone						-0.62	0.00	0.62	1.37	2.18
Corticosterone						-0.03	0.55	1.17	1.94	2.77
Toluene				-0.57	0.24	0.98	1.35	1.70		
Hexylfluorobenzene				-0.25	0.62	1.43	1.84	2.22		
Benzyl Alcohol							-0.77	-0.43	-0.09	0.22
o-Nitrophenol						0.26	0.58	0.90	1.18	
Propachlor				-0.90	-0.03	0.85	1.30	1.76		
1-Methyl naphthalene			-0.53	-0.08	0.80	1.77	2.27	2.79		
Tri-p-toyly phosphate	-1.79	-0.28	0.36	0.91	2.08	3.63				
Methyl benzyl amine					-0.72	0.00	0.32	0.65	1.01	
Fluorobenzeze				-0.84	-0.03	0.71	1.03	1.35		
Chloropropham				-0.33	0.62	1.60	2.09	2.63		
Dimethyl phthalate					-0.50	0.27	0.65	1.06	1.49	
Diethyl phthalate					0.23	1.09	1.55	2.04	2.58	
Dibutyl phthalate			0.07	0.56	1.63	2.83	3.54			
1-Methyl phenanthrene			-0.16	0.34	1.35	2.45	3.10			
Butyl benzyl phthalate			0.02	0.55	1.65	2.93	3.68			
Dioctyl phthalate	-0.12	1.16	1.78	2.45	4.03					
p-Terphenyl		-0.36	0.27	0.78		3.28				
Chrysene		-0.48	0.09	0.55	1.63					
1,1,4,4,-Teraphenyl-1,3-butadiene	-0.59	0.71	1.34	1.92	3.37					
Octadecanophenone	0.55	1.88	2.56	3.28						

Column: Zorbax ODS
Mobile phase: MeOH/water
Reference: This work

Solute	ln k' at volume % of methanol in the mobile phase							
	80	75	70	65	60	50	45	40
Methyl Paraben			-1.25	-0.81		0.59	1.01	1.46
Ethyl Paraben			-0.56	-0.10		1.35	1.81	
Propyl Paraben		-0.46	0.12	0.59		2.15		
Butyl Paraben		0.14	0.72	1.24		2.95		
Cortisone				-0.10	0.45	1.62	2.23	
Corticosterone			0.35	0.86	1.41	2.60		
Toluene			1.15	1.57	1.96	2.76		
Hexylfluorobenzene		0.77	1.26	1.72	2.16	3.00		
Benzyl Alcohol					-0.54	0.17	0.50	0.84
o-Nitrophenol		-0.67	-0.17		0.57	1.24		
Propachlor	-0.86	-0.29	0.29		1.26	2.26		
1-Methyl naphthalene	1.11	1.62	2.17	2.64	3.20			
Tri-p-toyly phosphate	1.66	2.49	3.36	4.21				
Methyl benzyl amine		-0.90	-0.43	-0.06	0.33	1.12	1.51	
Fluorobenzeze		0.01	0.45	0.83	1.23	1.99		
Chloropropham		0.32	0.89	1.42	1.98	3.12		
Dimethyl phthalate					0.23	1.13	1.62	2.12
Diethyl phthalate		-0.25	0.31	0.78	1.32	2.37		
Dibutyl phthalate	1.00	1.68	2.35	3.04				
1-Methyl phenanthrene	1.97	2.57	3.18	3.78				
Butyl benzyl phthalate	0.92	1.61	2.31	3.01				

Column: Zorbax C-8
Mobile phase: MeOH/water
Reference: This work

Solute	ln k' at volume % of methanol in the mobile phase							
	80	75	70	65	60	50	45	40
Methyl Paraben					-0.35	0.61	1.06	1.52
Ethyl Paraben					0.38	1.40	1.88	2.38
Propyl Paraben			-0.03		1.08	2.20	2.74	
Butyl Paraben			0.56		1.77	3.00	3.60	
Cortisone					0.15	1.46	2.01	2.71
Corticosterone			-0.12	0.47	1.09	2.38		
Toluene			0.70	1.14	1.59	2.49		
Hexylfluorobenzene			0.99	1.47	1.96	2.91		
Benzyl Alcohol					-0.73	0.12	0.47	0.83
o-Nitrophenol			-0.51	-0.09	0.29	1.07		
Propachlor			0.12	0.64	1.19	2.28		
1-Methyl naphthalene			1.54	2.07	2.66	3.83		
Tri-p-toyly phosphate	1.36	2.26	3.19	4.13				
Methyl benzyl amine				-0.38	0.06	0.93	1.31	
Fluorobenzeze			0.16	0.55	0.98	1.81		
Chloropropham			0.86	1.40	2.01	3.25		
Dimethyl phthalate				-0.53	0.06	1.09	1.55	
Diethyl phthalate			0.13	0.67	1.26	2.47		
Dibutyl phthalate	0.79	1.51	2.25	2.97	3.83			
1-Methyl phenanthrene	1.05	1.68	2.31	2.96				
Butyl benzyl phthalate	0.66	1.40	2.16	2.90	3.78			
p-Terphenyl	1.45	2.16	2.94	3.73				
Chrysene	1.28	1.94	2.64	3.36				

Column: Zorbax phenyl
Mobile phase: MeOH/water
Reference: This work

Solute	ln k' at volume % of methanol in the mobile phase							
	80	70	65	60	55	50	45	40
Methyl Paraben				-0.39	0.06	0.50	0.92	1.35
Ethyl Paraben				0.14	0.61	1.05	1.55	
Propyl Paraben		-0.49		0.70	1.21	1.69		
Butyl Paraben		0.01		1.26	1.81	2.35		
Cortisone			0.45	1.02	1.54		2.66	
Corticosterone		0.62	1.16	1.75	2.31			
Toluene			0.33	0.80	1.19	1.58		
Hexylfluorobenzene		0.33	0.82	1.30	1.71			
Benzyl Alcohol					-0.53	-0.17	0.17	0.50
o-Nitrophenol		-0.81	-0.36		0.43	0.76	1.10	
Propachlor		0.21	0.71		1.73	2.17		
1-Methyl naphthalene	-0.28	0.80	1.35		2.46			
Tri-p-toyly phosphate	1.07	2.79	3.75					
Methyl benzyl amine				0.12	0.49	0.81	1.22	
Fluorobenzeze				0.36	0.73	1.05	1.42	
Chloropropham		0.25		1.47	2.04	2.58		
Dimethyl phthalate		-0.65		0.33		1.18	1.66	
Diethyl phthalate		0.12	0.63	1.21		2.22		
Dibutyl phthalate	0.37	1.75	2.48	3.28				
1-Methyl phenanthrene	0.41	1.63	2.25	2.99				
Butyl benzyl phthalate	0.56	1.98	2.74	3.59				

Column: Zorbax TMS
Mobile phase: MeOH/water
Reference: This work

Solute	ln k' at volume % of methanol in the mobile phase									
	80	70	65	60	55	50	45	40	35	30
Methyl Paraben					-0.86	-0.33	0.15	0.55	0.95	1.34
Ethyl Paraben					-0.19	0.30	0.81	1.26	1.72	
Propyl Paraben					0.38	0.88	1.46	1.98		
Butyl Paraben			-0.39		0.91	1.43	2.08	2.70		
Cortisone					-0.45	0.23	0.76	1.48		
Corticosterone			-1.03			0.88	1.39	2.16		
Toluene					0.13	0.63	0.96	1.32		
Hexylfluorobenzene			-0.28	0.21		1.02	1.35	1.66		
Benzyl Alcohol						-1.21	-0.89	-0.47	-0.18	0.08
o-Nitrophenol						-0.36	0.07	0.47	0.72	1.10
Propachlor					0.47	1.12	1.57	2.11		
1-Methyl naphthalene			-0.04	0.57		1.64	2.12	2.69		
Tri-p-toyly phosphate	-0.60	1.14	2.02	2.93						
Methyl benzyl amine				-0.90		0.02	0.37	0.81	1.17	
Fluorobenzeze				-0.57		0.28	0.58	0.91	1.17	
Chloropropham			-0.23	0.41		1.53	2.03	2.68		
Dimethyl phthalate				-0.76		0.32	0.80	1.29	1.71	
Diethyl phthalate				0.11		1.28	1.83	2.41		
Dibutyl phthalate		0.45	1.09	1.86	2.65					
1-Methyl phenanthrene	-1.51	0.05		1.31		2.70	3.42			
Butyl benzyl phthalate		0.35	1.04	1.82	2.64					

Column: Zorbax cyano
Mobile phase: MeOH/water
Reference: This work

Solute	ln k' at volume % of methanol in the mobile phase										
	80	70	65	60	55	50	45	40	35	30	25
Methyl Paraben						-0.47	-0.02		0.74	1.05	1.3503
Ethyl Paraben						-0.04	0.43		1.28	1.64	
Propyl Paraben				-0.55		0.41	0.92		1.89		
Butyl Paraben				-0.19		0.85	1.41		2.51		
Cortisone							0.34	0.82	1.34	1.83	
Corticosterone							0.77	1.27	1.80	2.32	
Toluene				-0.36			0.76	1.08	1.41	1.67	
Hexylfluorobenzene				-0.06			0.99	1.29	1.56	1.83	
Benzyl Alcohol						-1.35		-0.65	-0.34	-0.12	0.09
o-Nitrophenol						-0.03		0.52	0.83	1.07	
Propachlor						0.28		1.20	1.66	2.18	
1-Methyl naphthalene				0.64	1.12	1.61		2.57	3.06		
Tri-p-toyly phosphate	-1.55	0.16	0.91	1.69	2.49	3.38					
Methyl benzyl amine				-1.17		-0.41	0.00		0.74	1.06	1.36
Fluorobenzeze				-0.61		0.13	0.49		1.10	1.33	
Chloropropham				0.20		1.21	1.71		2.74		
Dimethyl phthalate					-0.65	-0.23		0.64		1.47	
Diethyl phthalate				-0.51	-0.01	0.48		1.48			
Dibutyl phthalate		-0.49	0.15		1.43		2.79				
1-Methyl phenanthrene		0.42	0.97	1.57	2.17	2.79					
Butyl benzyl phthalate		-0.15	0.48		1.80		3.24				

Column: Ultrasphere C-8
Mobile phase: MeOH/water
Reference: This work

Solute	ln k' at volume % of methanol in the mobile phase						
	80	70	65	60	50	45	40
Methyl Paraben			-0.59	-0.20	0.65	1.09	1.51
Ethyl Paraben			-0.03	0.39	1.36	1.86	
Propyl Paraben			0.57	1.05	2.12	2.69	
Butyl Paraben			1.15	1.69	2.91	3.54	
Cortisone				0.35	1.50	2.15	2.77
Corticosterone			0.66	1.20	2.40	3.07	
Toluene		0.72	1.12	1.52	2.77		
Hexylfluorobenzene		0.99	1.44	1.89	3.20		
Benzyl Alcohol				-0.42	0.26	0.59	0.89
o-Nitrophenol			-0.05	0.31	1.03	1.39	
Propachlor		0.19	0.66	1.14	2.18	2.74	
1-Methyl naphthalene	0.49	1.49	2.01	2.53			
Tri-p-toyly phosphate	1.31	3.03	3.97	4.97			
Methyl benzyl amine		-0.59	-0.24	0.12	0.88	1.27	
Fluorobenzeze		0.17	0.56	0.94	1.70	2.08	
Chloropropham		0.82	1.36	1.93	3.11		
Dimethyl phthalate			-0.32	0.07	1.03	1.52	
Diethyl phthalate	-0.73	0.17	0.68	1.17	2.31		
1-Methyl phenanthrene	1.03	2.20	2.83	3.47			

Column: B & J C-8
Mobile phase: MeOH/water
Reference: This work

Solute	ln k' at volume % of methanol in the mobile phase							
	80	70	65	60	50	45	40	35
Methyl Paraben			-0.61	-0.24	0.59	0.98	1.37	
Ethyl Paraben			-0.18	0.23	1.13	1.58	2.03	
Propyl Paraben			0.25	0.70	1.70	2.22		
Butyl Paraben			0.67	1.18	2.29	2.87		
Cortisone			-0.46	0.00	1.03	1.58		
Corticosterone			0.20	0.69	1.78	2.35		
Toluene			0.39	0.75	1.49	1.83		
Hexylfluorobenzene			0.50	0.88	1.64	1.96		
Benzyl Alcohol				-0.79	-0.13	0.12	0.42	0.66
o-Nitrophenol				0.03	0.66	0.96	1.28	
Propachlor			0.23	0.66	1.60	2.05	2.55	
1-Methyl naphthalene	-0.24	0.73	1.24	1.73	2.74	3.23		
Tri-p-toyly phosphate	0.46	2.04	2.93	3.81				
Methyl benzyl amine				0.02		1.06	1.44	1.79
Fluorobenzeze			0.01	0.32		1.32	1.64	
Chloropropham		0.26	0.80	1.30		2.90		
Dimethyl phthalate		-0.86	-0.35	-0.01	0.86		1.75	
Diethyl phthalate		-0.18	0.34	0.79	1.82		2.88	
1-Methyl phenanthrene	0.41	1.53	2.15	2.72	4.02			

Column: B & J C-18
Mobile phase: MeOH/water
Reference: This work

Solute	ln k' at volume % of methanol in the mobile phase							
	80	75	70	65	60	50	45	40
Methyl Paraben				-0.40	-0.03	0.78	1.19	1.64
Ethyl Paraben				0.18	0.61	1.52	1.99	2.50
Propyl Paraben		-0.13		0.81	1.28	2.32		
Butyl Paraben		0.39		1.44	1.97	3.14		
Cortisone				0.10	0.61	1.73	2.39	
Corticosterone		-0.06	0.44	0.94	1.49	2.65		
Toluene		0.85	1.26	1.67	2.06	2.85		
Hexylfluorobenzene		0.92	1.38	1.84	2.27	3.09		
Benzyl Alcohol					-0.29	0.36	0.66	0.99
o-Nitrophenol		-0.25	0.11	0.43	0.77	1.34	1.79	
Propachlor		0.06	0.52	0.97	1.45	2.45		
1-Methyl naphthalene	1.24	1.74	2.26	2.77	3.29	4.38		
Methyl benzyl amine		-0.48		0.20	0.55	1.30	1.70	
Fluorobenzeze		0.24		1.01	1.39	2.14		
Chloropropham	-0.17	0.55		1.60	2.16	3.31		
Dimethyl phthalate			-0.39	0.03		1.31	1.79	
Diethyl phthalate	-0.19	0.08	0.54	1.04		2.60		
1-Methyl phenanthrene	2.38	2.72	3.33	3.97				

Column: High Density (Sentell 1987)
Mobile phase: MeOH/water
Reference: This work

Solute	ln k' at volume % of methanol in the mobile phase							
	80	70	65	60	50	45	40	35
Methyl Paraben				-0.08	0.62	1.01	1.43	1.85
Ethyl Paraben				0.52	1.34	1.81	2.29	
Propyl Paraben		0.25	0.69	1.17	2.13			
Butyl Paraben		0.81	1.32	1.86	2.94			
Cortisone				0.45	1.48	2.09	2.74	
Corticosterone			0.78	1.29	2.39	3.02		
Benzyl Alcohol					0.26	0.56	0.86	1.18
o-Nitrophenol			0.22	0.53	1.09	1.41		1.96
Propachlor		0.24	0.69	1.17	2.09	2.60		
1-Methyl naphthalene	1.16	2.07	2.57	3.09	4.11			
Methyl benzyl amine				0.27	0.93	1.29	1.66	
Fluorobenzeze								
Chloropropham	0.07	0.98	1.51			3.70		
Dimethyl phthalate				0.19	0.97	1.43	1.90	
Diethyl phthalate			0.76	1.23	2.23	2.80		

APPENDIX B
SLOPES FROM EQUATION 3-1 AND ln k_w

Column: Zorbax ODS
Mobile phase: ACN/water
Reference: This work

Solute	Slope from equation 3-1	ln k_w
Methyl Paraben	0.95	5.14
Ethyl Paraben	1.21	7.56
Propyl Paraben	1.25	8.09
Butyl Paraben	1.39	9.52
Cortisone	1.39	7.82
Corticosterone	1.28	8.08
Toluene	1.06	8.50
Hexylfluorobenzene	1.39	10.22
Benzyl Alcohol	0.65	3.25
o-Nitrophenol	0.98	6.66
Propachlor	1.19	8.40
1-Methyl naphthalene	1.38	11.39
Tri-p-toyly phosphate	2.00	16.22
Methyl benzyl amine	1.03	6.30
Fluorobenzeze	1.20	8.63
Chloropropham	1.49	10.93
Dimethyl phthalate	1.41	8.99
Diethyl phthalate	1.41	9.89
Dibutyl phthalate	1.61	12.91
1-Methyl phenanthrene	1.50	12.77
Butyl benzyl phthalate	1.70	13.69
Dioctyl phthalate	1.89	18.28
p-Terphenyl	1.66	14.76
Chrysene	1.49	13.23

Column: Zorbax C-8
Mobile phase: ACN/water
Reference: This work

Solute	Slope from equation 3-1	$\ln k_w$
Methyl Paraben	1.28	7.18
Ethyl Paraben	1.24	7.46
Propyl Paraben	1.57	10.18
Butyl Paraben	1.56	10.55
Cortisone	1.56	8.05
Corticosterone	1.54	9.29
Toluene	1.27	9.15
Hexylfluorobenzene	1.36	10.43
Benzyl Alcohol	0.82	3.84
o-Nitrophenol	1.31	8.77
Propachlor	1.34	9.07
1-Methyl naphthalene	1.39	11.22
Tri-p-toyly phosphate	1.72	14.45
Methyl benzyl amine	1.27	8.25
Fluorobenzeze	1.25	8.69
Chloropropham	1.50	11.07
Dimethyl phthalate	1.40	8.75
Diethyl phthalate	1.38	9.79
Dibutyl phthalate	1.60	13.38
1-Methyl phenanthrene	1.43	12.15
Butyl benzyl phthalate	1.68	13.42
Dioctyl phthalate	2.20	20.84
p-Terphenyl	1.67	13.89
Chrysene	1.56	13.25

Column: Zorbax phenyl
Mobile phase: ACN/water
Reference: This work

Solute	Slope from equation 3-1	ln k_w
Methyl Paraben	0.81	4.68
Ethyl Paraben	0.92	5.60
Propyl Paraben	1.07	7.23
Butyl Paraben	1.20	7.93
Cortisone	1.00	5.72
Corticosterone	0.99	6.36
Toluene	0.92	6.66
Hexylfluorobenzene	1.04	7.63
Benzyl Alcohol	0.56	2.87
o-Nitrophenol	0.81	5.33
Propachlor	0.98	6.80
1-Methyl naphthalene	1.20	9.33
Tri-p-toyly phosphate	1.60	12.98
Methyl benzyl amine	0.79	5.02
Fluorobenzeze	0.90	6.33
Chloropropham	1.24	8.76
Dimethyl phthalate	0.87	5.65
Diethyl phthalate	1.01	7.14
Dibutyl phthalate	1.44	11.08
1-Methyl phenanthrene	1.40	10.85
Butyl benzyl phthalate	1.77	13.00
Dioctyl phthalate	2.11	17.16
p-Terphenyl	1.81	14.23
Chrysene	1.67	12.89
1,1,4,4,-Tetraphenyl-1,3-butadiene	2.12	17.79
Octadecanophenone	2.47	20.88

Column: Zorbax TMS
Mobile phase: ACN/water
Reference: This work

Solute	Slope from equation 3-1	$\ln k_w$
Methyl Paraben	1.01	5.24
Ethyl Paraben	1.02	5.97
Propyl Paraben	1.17	6.94
Butyl Paraben	1.28	8.18
Cortisone	1.18	6.07
Corticosterone	1.19	6.90
Toluene	1.03	6.60
Hexylfluorobenzene	1.12	7.58
Benzyl Alcohol	0.51	1.89
o-Nitrophenol	0.59	3.68
Propachlor	1.21	7.56
1-Methyl naphthalene	1.31	9.17
Tri-p-toyly phosphate	2.20	15.84
Methyl benzyl amine	0.77	4.25
Fluorobenzeze	0.99	6.11
Chloropropham	1.34	8.97
Dimethyl phthalate	0.90	5.24
Diethyl phthalate	1.06	7.00
Dibutyl phthalate	1.71	12.52
1-Methyl phenanthrene	1.60	11.38
Butyl benzyl phthalate	1.81	13.23
Dioctyl phthalate	2.39	18.83
p-Terphenyl	1.95	14.06
Chrysene	1.72	12.55
1,1,4,4,-Tetraphenyl-1,3-butadiene	2.27	17.26
Octadecanophenone	2.52	21.04

Column: Zorbax cyano
Mobile phase: ACN/water
Reference: This work

Solute	Slope from equation 3-1	$\ln k_w$
Methyl Paraben	0.67	3.18
Ethyl Paraben	0.72	3.88
Propyl Paraben	1.04	5.83
Butyl Paraben	1.14	6.95
Cortisone	0.93	4.39
Corticosterone	0.95	5.08
Toluene	0.92	5.73
Hexylfluorobenzene	1.12	7.18
Benzyl Alcohol	0.38	1.19
o-Nitrophenol	0.57	3.40
Propachlor	0.94	5.51
1-Methyl naphthalene	1.21	8.26
Tri-p-toyly phosphate	2.11	14.16
Methyl benzyl amine	0.52	2.69
Fluorobenzeze	0.72	4.33
Chlorcpropham	1.13	7.21
Dimethyl phthalate	0.99	5.28
Diethyl phthalate	1.10	6.72
Dibutyl phthalate	2.04	13.74
1-Methyl phenanthrene	1.73	11.68
Butyl benzyl phthalate	1.60	11.28
Dioctyl phthalate	2.55	17.93
p-Terphenyl	1.90	13.91
Chrysene	1.75	12.54
1,1,4,4,-Tetraphenyl-1,3-butadiene	2.34	16.68
Octadecanophenone	2.69	19.77

Column: Zorbax ODS
Mobile phase: MeOH/water
Reference: This work

Solute	Slope from equation 3-1	$\ln k_w$
Methyl Paraben	1.19	6.00
Ethyl Paraben	1.32	7.91
Propyl Paraben	1.49	9.23
Butyl Paraben	1.62	10.84
Cortisone	1.56	9.25
Corticosterone	1.55	10.02
Toluene	1.10	8.02
Hexylfluorobenzene	1.30	9.54
Benzyl Alcohol	0.82	4.11
o-Nitrophenol	1.09	6.29
Propachlor	1.59	10.34
1-Methyl naphthalene	1.87	14.02
Tri-p-toyly phosphate	3.15	22.80
Methyl benzyl amine	1.14	6.85
Fluorobenzeze	1.15	7.68
Chloropropham	1.63	10.87
Dimethyl phthalate	1.12	6.78
Diethyl phthalate	1.53	10.06
Dibutyl phthalate	2.51	18.41
1-Methyl phenanthrene	2.23	16.74
Butyl benzyl phthalate	2.58	18.82

Column: Zorbax C-8
Mobile phase: MeOH/water
Reference: This work

Solute	Slope from equation 3-1	ln k_w
Methyl Paraben	1.11	5.95
Ethyl Paraben	1.19	7.10
Propyl Paraben	1.51	9.50
Butyl Paraben	1.66	10.96
Cortisone	1.51	8.60
Corticosterone	1.72	10.95
Toluene	1.23	8.53
Hexylfluorobenzene	1.31	9.07
Benzyl Alcohol	0.93	4.53
o-Nitrophenol	1.07	6.13
Propachlor	1.48	9.40
1-Methyl naphthalene	1.57	11.18
Tri-p-toyly phosphate	3.41	24.21
Methyl benzyl amine	1.14	6.65
Fluorobenzeze	1.13	7.01
Chloropropham	1.65	11.43
Dimethyl phthalate	1.39	7.92
Diethyl phthalate	1.61	10.41
Dibutyl phthalate	2.73	19.29
1-Methyl phenanthrene	2.35	17.31
Butyl benzyl phthalate	2.80	19.71
p-Terphenyl	2.82	20.97
Chrysene	2.57	18.19

Column: Zorbax phenyl
Mobile phase: MeOH/water
Reference: This work

Solute	Slope from equation 3-1	$\ln k_w$
Methyl Paraben	1.03	5.50
Ethyl Paraben	1.18	6.87
Propyl Paraben	1.49	8.73
Butyl Paraben	1.61	10.41
Cortisone	1.49	9.13
Corticosterone	1.69	11.16
Toluene	1.09	6.79
Hexylfluorobenzene	1.37	8.96
Benzyl Alcohol	0.80	3.67
o-Nitrophenol	1.06	6.00
Propachlor	1.37	8.66
1-Methyl naphthalene	1.81	12.23
Methyl benzyl amine	0.91	5.20
Fluorobenzeze	0.88	5.33
Chloropropham	1.60	10.48
Dimethyl phthalate	1.25	7.39
Diethyl phthalate	1.44	9.08
Dibutyl phthalate	2.62	18.35
1-Methyl phenanthrene	2.31	15.78
Butyl benzyl phthalate	2.72	18.66

Column: Zorbax TMS
Mobile phase: MeOH/water
Reference: This work

Solute	Slope from equation 3-1	$\ln k_w$
Methyl Paraben	1.11	5.25
Ethyl Paraben	1.16	6.11
Propyl Paraben	1.25	6.99
Butyl Paraben	1.56	8.95
Cortisone	1.47	6.97
Corticosterone	1.62	8.44
Toluene	0.91	4.97
Hexylfluorobenzene	0.97	5.61
Benzyl Alcohol	0.86	2.96
o-Nitrophenol	0.93	4.09
Propachlor	1.25	7.09
1-Methyl naphthalene	1.36	8.33
Tri-p-toyly phosphate	3.18	20.69
Methyl benzyl amine	1.01	4.94
Fluorobenzeze	0.85	4.34
Chloropropham	1.44	8.38
Dimethyl phthalate	1.21	6.26
Diethyl phthalate	1.37	8.16
Dibutyl phthalate	2.21	14.47
1-Methyl phenanthrene	2.08	13.27
Butyl benzyl phthalate	2.29	14.52

Column: Zorbax cyano
Mobile phase: MeOH/water
Reference: This work

Solute	Slope from equation 3-1	$\ln k_w$
Methyl Paraben	0.87	3.79
Ethyl Paraben	1.10	5.12
Propyl Paraben	1.22	6.49
Butyl Paraben	1.35	7.70
Cortisone	1.29	5.81
Corticosterone	1.35	6.90
Toluene	0.86	4.56
Hexylfluorobenzene	0.80	4.52
Benzyl Alcohol	0.70	2.17
o-Nitrophenol	0.74	3.46
Propachlor	1.25	5.99
1-Methyl naphthalene	1.17	7.34
Tri-p-toyly phosphate	2.50	15.78
Methyl benzyl amine	0.88	3.83
Fluorobenzeze	0.83	4.11
Chloropropham	1.26	7.12
Dimethyl phthalate	1.09	4.99
Diethyl phthalate	1.16	6.21
Dibutyl phthalate	1.84	11.12
1-Methyl phenanthrene	1.65	10.83
Butyl benzyl phthalate	1.91	12.54

Column: Ultrasphere C-8
Mobile phase: MeOH/water
Reference: This work

Solute	Slope from equation 3-1	$\ln k_w$
Methyl Paraben	1.07	6.03
Ethyl Paraben	1.27	7.55
Propyl Paraben	1.43	9.35
Butyl Paraben	1.56	10.45
Cortisone	1.44	8.38
Corticosterone	1.61	10.31
Toluene	1.42	9.42
Hexylfluorobenzene	1.53	10.26
Benzyl Alcohol	0.78	4.03
o-Nitrophenol	0.96	5.65
Propachlor	1.40	8.95
1-Methyl naphthalene	1.84	13.12
Tri-p-toyly phosphate	3.30	24.26
Methyl benzyl amine	1.02	5.57
Fluorobenzeze	1.05	6.97
Chloropropham	1.57	10.98
Dimethyl phthalate	1.25	7.39
Diethyl phthalate	1.56	9.85
1-Methyl phenanthrene	2.20	15.84

Column: B & J C-8
Mobile phase: MeOH/water
Reference: This work

Solute	Slope from equation 3-1	ln k_w
Methyl Paraben	1.01	5.74
Ethyl Paraben	1.12	6.58
Propyl Paraben	1.33	8.44
Butyl Paraben	1.48	9.50
Cortisone	1.37	7.66
Corticosterone	1.45	8.91
Toluene	0.97	6.13
Hexylfluorobenzene	0.99	6.43
Benzyl Alcohol	0.71	3.24
o-Nitrophenol	0.74	4.20
Propachlor	1.17	7.04
1-Methyl naphthalene	1.48	9.80
Tri-p-toyly phosphate	3.02	20.59
Methyl benzyl amine	0.86	4.92
Fluorobenzeze	0.83	4.96
Chloropropham	1.47	9.77
Dimethyl phthalate	1.10	6.22
Diethyl phthalate	1.30	7.84
1-Methyl phenanthrene	1.85	12.75

Column: B & J C-18
Mobile phase: MeOH/water
Reference: This work

Solute	Slope from equation 3-1	$\ln k_w$
Methyl Paraben	1.03	5.80
Ethyl Paraben	1.17	7.44
Propyl Paraben	1.45	9.11
Butyl Paraben	1.63	11.17
Cortisone	1.53	9.46
Corticosterone	1.59	10.34
Toluene	1.17	8.74
Hexylfluorobenzene	1.26	9.12
Benzyl Alcohol	0.76	3.97
o-Nitrophenol	0.95	6.04
Propachlor	1.40	9.15
1-Methyl naphthalene	1.62	12.14
Methyl benzyl amine	1.05	6.27
Fluorobenzeze	1.12	7.48
Chloropropham	1.78	12.34
Dimethyl phthalate	1.20	7.23
Diethyl phthalate	1.46	9.64
1-Methyl phenanthrene	2.00	15.22

Column: High density (Sentell 1987)
Mobile phase: MeOH/water
Reference: This work

Solute	Slope from equation 3-1	$\ln k_w$
Methyl Paraben	0.94	5.22
Ethyl Paraben	1.05	6.67
Propyl Paraben	1.29	8.41
Butyl Paraben	1.46	9.94
Cortisone	1.34	6.97
Corticosterone	1.50	9.87
Benzyl Alcohol	0.74	3.90
o-Nitrophenol	0.75	4.77
Propachlor	1.29	8.41
1-Methyl naphthalene	1.52	11.53
Methyl benzyl amine	0.82	4.94
Chloropropham	1.58	10.91
Dimethyl phthalate	1.01	5.64
Diethyl phthalate	1.36	9.03

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BIOGRAPHICAL SKETCH

Peter Tai Yuen Ying was born on February 28, 1962, in Hong Kong. He completed his high school education in Hong Kong, and at the age of 19, he came over to America to further his education. He first attended the University of Wisconsin-Madison. Due to numerous reasons, and one being the dislike of long winters, he transferred to the College of Charleston in South Carolina in June 1983. He later graduated with a B.S. degree in chemistry in May 1985 from the College of Charleston. He entered the graduate school at the University of Florida in August 1985. After all these years of living and learning in America, he has become fascinated by the western cuisines, music and women (but not necessarily in that order). Therefore, upon completion of the requirements for the degree of Doctor of Philosophy, he will join the Wesley-Jessen Corp. in Chicago as a research chemist so he can continue to pursue his fascinations.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John G. Dorsey, Chair
Associate Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the Department of Chemistry in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requiremnets for the degree of Doctor of Philosophy.

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